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THE TENACITY OF THE VIRUS OF FOOT-AND-MOUTH DISEASE UNDER FIELD CONDITIONS.

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DURING recent years the tenacity of the virus of foot-and-mouth disease under widely different conditions has been tested in a number of European Laboratories, and recent veterinary literature contains abundant information as to the viability of the virus under conditions which at times faithfully represent natural conditions or conditions met with in practice, whilst in others they merely approximate to natural conditions. The history of foot-and-mouth disease in Great Britain during the past 20 years presents an unique opportunity for examining the question of the tenacity of virus under field or natural conditions, inasmuch as the stamping out policy has been in force during that period as a general policy which has been departed from in a limited number of cases only. The observations are, therefore, not liable to be confounded by the presence of recovered animals in the herds, and recurrence of disease is not to be associated with the presence of possible "carriers" of infection, nor, generally speaking, is the risk of infection from other centres of disease comparable with that which would arise in countries where the disease is dealt with by isolation.

Before proceeding to the examination of the cases in which the survival of the virus was demonstrated by the reappearance of disease among animals after an interval, it will be necessary to describe briefly the measures taken on the diagnosis of disease.

On declaration of the disease the affected animals and those in contact are valued and within 12 to 24 hours are slaughtered and their carcases incinerated. From the earliest moment buildings,

yards, pig-sties, etc., are sprayed continuously with a disinfectant solution, and after the carcases have been disposed of a thorough cleansing and disinfection of the infected buildings is carried out, a solution of a coal-tar preparation being used.

Heavily contaminated food materials, litter, and sacks are burnt.

The clothing of attendants is fumigated, and vehicles, milk churns, threshing machines, etc., are disinfected by suitable processes.

Manure is closely packed into a heap 4 to 6 feet high and fermentation is relied on to destroy the virus except on the surface, which is sprayed with disinfectant solution.

The outside of hay-ricks, straw-ricks, etc., is sprayed with formaldehyde solution. Fumigation with formaldehyde in a closed chamber is carried out where it is necessary to deal with cereals or quantities of artificial feeding-stuffs which have been exposed to lesser risk of infection. Formerly fumigation by sulphur was employed, but for some years only formaldehyde has been used for this purpose. In the treatment of cake and grain formaldehyde gas has been found to be superior to spraying with a watery solution, which is likely to be followed after the lapse of a few weeks by abundant growth of moulds.

Time and natural agencies alone are depended upon in the case of pastures, since disinfection is not practicable and only the heavily contaminated parts, such as gateways and the vicinity of the incineration pit, are disinfected.

Then, after an interval animals, are re-admitted to the premises.

In certain cases this interval between the final cleansing and disinfecting of the premises and re-opening must be modified—for example, where disease has occurred in an abattoir or in one of the landing places at the ports at which animals are imported. On such premises disinfection is carried out comparatively rapidly and does not present the problems which one finds on farm premises. Generally, when the disinfection has been completed these premises are immediately re-opened to trade, though under control since general restrictions are in force in the infected area. Despite the fact that no interval of time is usually insisted upon between completion of disinfection and resumption of trade, in only one out of about 200 such cases has disease reappeared.

At present the normal practice is to allow animals to be brought on to infected farms six weeks after disinfection has been completed, or alternatively eight weeks after slaughter of the diseased animals, whichever period is the shorter. The number of animals permitted to be brought on to the infected premises at this stage is 50 per cent. of the original stock. These animals are kept under observation for three weeks, and if at the expiration of this period they remain healthy all the restrictions are removed. This procedure has been slightly modified from time to time in respect of the number of animals which owners are allowed to bring on, but the accompanying table gives details of the time which elapsed

between the completion of slaughter, the completion of disinfection, and the recurrence of disease, and it also shows the length of time during which animals were on the premises before they became affected. Re-stocking took place at varying intervals from 62—176 days after slaughter of the original diseased stock, and of 28—128 days after disinfection on the premises; and the newly arrived animals developed foot-and-mouth disease at intervals varying from 6—104 days after arrival on the premises.

The records of outbreaks of foot-and-mouth disease in Great Britain during the past 20 years (1909-1928) show that there occurred 5,659 outbreaks, in 105 of which the animals were dealt with by isolation instead of by the usual procedure of slaughter. In other words, the slaughter policy was adopted in 5,554 cases. Of the 5,554 premises on which animals were slaughtered, disease re-appeared on 57. Of these, there is reason to think that in 13 instances disease was brought on with the animals with which the farms were re-stocked during times when disease was prevalent in the districts, despite the care taken to obviate this risk, or infection was re-introduced by other means from outside sources. Details are appended on next page of 42 of the remaining cases.

A further set of premises on which disease recurred was a pig market, and in this case the survival of virus was attributed to the difficulty of thorough disinfection on account of faulty woodwork of a section of the premises. After the recurrence of disease the woodwork was removed, and no further trouble occurred.

In another case, which does not appear in the table, the circumstances were unusual. An outbreak of disease occurred in a flock of sheep hurdled on a crop of turnips. Two months earlier disease had occurred on another part of the farm among cattle which had been slaughtered, and the usual methods of disinfection had been employed on the infected part of the premises. On declaration of disease among the sheep it was ascertained that the cattle which had been affected in the earlier outbreak had, on one occasion just before the outbreak was reported, broken into this turnip field and were there for some little while, during which they ate some of the growing turnips, both roots and foliage. The sheep had been in the field about three weeks before they developed disease, but in accordance with the custom in this country they were penned on a fresh patch of turnips daily, and it was not until some days after their admission to the field that they reached the part to which the cattle had had access.

It is estimated that pastures are involved in about 75 per cent. of the outbreaks which occur in this country, but in no instance has disease recurred where pastures alone were involved in an outbreak. The minimum period for re-stocking pastures has never been less than 6 weeks.

All the recurrences appear to be associated with buildings or with foodstuffs and straw. In four cases it was reported that defective floors had probably been responsible for the upkeep of

No. of Case.	Interval which elapsed before Reappearance of Disease after :		
	(a) Re-stocking.	(b) Slaughter of the Affected Animals.	(c) Disinfection of the Premises, etc.
	Days.	Days.	Days.
1	22	89	66
2	25	108	107
3	43	96	—
4	26	90	66
5	14	93	71
6	44	140	128
7	35	92	68
8	10	96	76
9	37	99	69
10	7	80	62
11	6	63	50
12	—	96	70
13	35	99	—
14	7	70	65
15	60	97	58
16	21	55	28
17	32	87	52
18	18	82	67
19	11	69	57
20	15	100	91
21	21	98	84
22	45	100	87
23	41	106	87
24	81	135	126
25	18	90	89
26	33	88	77
27	34	103	84
28	14	87	71
29	8	77	64
30	8	94	75
31	18	80	67
32	34	91	77
33	10	84	73
34	27	89	74
35	12	62	58
36	104	176	141
37	29	99	81
38	7	63	52
39	9	75	73
40	—	62	57
41	20	73	66
42	6	64	53
Average No. of days	26	90	74

virus despite the fact that disinfectant solutions had been used on a very liberal scale on account of the unsatisfactory hygienic conditions. The foodstuffs in most cases consisted of a variety of provender, including hay, meals, cake, and roots, and there were usually more or less abundant supplies of straw. In some cases the hay was stored in a loft over the cowshed. Such supplies of hay were contaminated by the attendants during the early stages of an outbreak, and since the lofts were not airtight fumigation was

impracticable. Destruction was equally impracticable in view of the large quantities frequently found in these lofts. The usual practice was to spray with a formalin solution the parts of the store to which the attendants had access in drawing supplies for the cattle. In only one case no foodstuffs remained after the cleaning of the premises after the first outbreak of disease.

In case No. 6 on the list, where disease occurred 140 days after the slaughter of the original diseased animals, the recurrence was attributed to a stack of straw which had been thrashed shortly before the original case was reported but when disease existed among the animals. The straw was used for the bedding of animals subsequently brought on.

It happens occasionally that just prior to notification of an outbreak a stack of grain has been thrashed on the premises, all farm hands assisting. There is, in such cases, a grave risk that the straw which is re-stacked after thrashing has been contaminated by workers tending diseased animals. Obviously it is impossible to disinfect the straw thoroughly, and impracticable to destroy it. In such cases the outside of the stack is sprayed with a formalin solution and the owner forbidden to use the straw for a minimum period of three months. In a number of cases where this practice has been adopted no recurrence of disease has been experienced.

Of the 42 cases which appear in the table, disease first appeared in one case in October ; in one case in March ; in 3 cases in February; and in the remaining cases in November and December; but it is not suggested that in relation to the subject under discussion any conclusion should be drawn from this, since the winter months represent the period of greatest incidence of the disease in Great Britain, probably because the viability of the virus, whether on its introduction from abroad or originating from an outbreak in this country, is assisted by the lower temperature and the lack of sunshine. It is, however, during these months that farms are found to be most heavily stocked with hay, straw, cake, roots, etc., which makes it more difficult to carry out the precautionary measures of disinfection.

All disinfectants to be used in connection with operations carried out under the Diseases of Animals Acts must be approved by the Ministry of Agriculture and Fisheries, and this approval is subject to a biological (Chick-Martin) test, and the approved dilution in each case is equivalent to the bactericidal effect of a 5 per cent. solution of phenol. Practically all proprietary disinfectants at present approved are of the tar-acid type. It has been shown that a high degree of success has been attained in practice in disposing of the risk of infection of foot-and-mouth disease by the use of these coal tar preparations, employed admittedly liberally and in conjunction with a lapse of time before animals are again brought to the infected premises ; but in quite a number of instances the time factor has not operated and it has been necessary to rely entirely on the disinfecting power of these agents to render such

places as slaughter-houses and lairs safe after the disposal of diseased animals had been effected, so that facilities for the reopening of the premises to trade at the earliest possible moment could be granted. It is recognised that these coal tar preparations do not give results in the Laboratory comparable with other preparations in the destruction of the virus of foot-and-mouth disease, and that their destructive quality is bactericidal rather than viricidal, but nevertheless the results of their use in practice must be regarded as highly satisfactory.

The legal requirements which stipulate the use of approved disinfectants extend beyond the disinfection of premises infected by various scheduled diseases. They include the preventive measures of disinfection which are compulsorily carried out in the case of markets, lairs, railway loading stations, railway trucks, ships, vehicles, etc.

That preventive disinfection of railway trucks, for example, can, when thoroughly carried out, play a most useful part in the prevention of spread of disease is undeniable. Whether Authorities should base their preventive measures essentially against foot-and-mouth disease as constituting the greatest immediate danger to the flocks and herds of the country, or whether they should bear in mind the general risks involved in the transport of animals, will determine the type of disinfectant to be used. One cannot ignore the more or less serious risks of infection involved in the transport of animals, and it is suggested that the type of disinfectant to be recommended is one which will be the most suitable to deal with such varied types of parasites and organisms as those which are responsible for the acariases, fungus diseases like ringworm, and diseases such as tuberculosis, Johne's disease, contagious abortion, foot rot, and mastitis, in addition to foot-and-mouth disease. In this country at any rate, the low incidence of anthrax enables us to ignore this disease as one requiring special attention when considering the disinfection of means of transport.

The results which have been obtained in Great Britain in the disinfection of foot-and-mouth disease infected places over a long period seem to justify the recommendation that disinfectants of the coal tar type, essentially bactericidal agents, are eminently suitable for use against the virus of foot-and-mouth disease and therefore can be recommended for general use in the disinfection of vehicles, railway trucks, ships, lairs, and railway stations.

They are easily obtainable in most countries and comparatively cheap. They have, however, one serious disadvantage in that they taint meat and other foodstuffs.

THE INTRODUCTION INTO GREAT BRITAIN OF ANTHRAX INFECTION BY MEANS OF PRODUCTS OF ANIMAL ORIGIN.

By Sir Ralph Jackson,

Ministry of Agriculture and Fisheries.

The spread of anthrax by means of infected products of animal origin having been tabled as a subject for discussion during the 1929 Session of the Committee of the International Office, the Director of Epizootics suggested that it would be useful if a note could be submitted on the experience in Great Britain, based on certain details which are published annually in the Report of the Diseases of Animals Division of the Ministry of Agriculture and Fisheries, indicating the origins or possible origins of outbreaks of anthrax in the country. For convenience a table is reproduced covering the past ten years.

The information conveyed in the table must be read as a record of the circumstances attending the outbreaks and not as a table of origins which have been proved by laboratory experiment. It would be quite impracticable to endeavour to submit to laboratory proof suspected materials, whether artificial feeding stuffs or artificial manures, which are found on infected premises. Even a positive result in any given outbreak would not be conclusive evidence that the materials involved had introduced disease to the premises, since they might have been infected after arrival at the farm by the carcase of the animal which died of anthrax on the premises.

Having regard to the viability of the spores of anthrax, it is perhaps surprising to observe that eighty per cent. of the outbreaks which arise in Great Britain occur on premises upon which the disease has not existed within the previous five years and where it is therefore not unreasonable to assume that disease has been introduced from outside.

The use of artificial feeding stuffs appears with great frequency in association with outbreaks on non-infected premises. These feeding stuffs include linseed cake, cotton cake, and a variety of other kinds of cake—frequently compounded—which are the residues of the vegetable oil trade, and they include also a variety of meals prepared from peas, beans, and the cereals. On the other hand, the use of artificial manure of animal origin appears to a very much smaller extent than imported feeding stuffs. Perhaps one should not hastily assume that the importance of artificial manure of animal origin is to be assessed by the relative figures which appear in the table, since it is quite conceivable that an artificial manure might have been applied to non-fodder crops one or two seasons earlier and that a later fodder crop was capable of conveying infection to animals, but at the time when anthrax occurred so long an interval had elapsed since the application of the artificial manure to the land that the importance of this factor was overlooked by the

ANTHRAX.

Year.	Total Number of outbreaks.	No. of Outbreaks.		Possible Sources of Infection on Farms Free from Disease for Five Years.							
		On premises infected within the previous five years.	On non-infected premises.	Effluent from tanyards or other similar industrial undertakings contaminating streams, etc.	Feeding of infected carcases, offals, etc., to pigs.	Use of imported feeding stuffs.	Use of artificial manures of animal origin on the land.	Use of both imported feeding stuffs and artificial manures.	Use of shoddy as litter.	A recent death not reported, but not improbably anthrax.	No explanation available.
1919	239	60	179	12	2	107	8	4	—	8	34
1920	472	73	399	2	4	294	13	31	1	12	40
1921	495	75	420	15	2	268	41	40	1	4	49
1922	515	50	465	7	5	302	29	60	4	3	55
1923	724	113	611	5	1	395	23	100	—	8	79
1924	734	127	607	7	2	414	24	95	1	6	58
1925	669	124	545	9	2	338	18	103	4	2	69
1926	703	194	509	3	—	371	17	77	2	1	38
1927	425	102	323	7	1	223	11	44	—	1	36
1928	536	143	393	3	2	304	7	52	1	6	18
	5,512	1,061	4,451	70	21	3,016	191	606	14	51	476
				1·6	·5	67·8	4·3	13·7	·3	1·1	10·7%

Figures for 1928 are approximate only: six outbreaks are omitted from the columns, "Possible sources of infection," as they were not classifiable under any of the headings.

owners of the animals when enquiry was made concerning the outbreak.

It will be seen that effluent from tanyards or other similar industrial undertakings contaminating water courses is not a very serious factor in the introduction of anthrax in Great Britain, although there is a small number of farms in certain parts of the country upon which the disease occurs from time to time, to which infection was probably conveyed from industrial sources in the first instance, and either the premises have become permanently infected or infection is re-introduced from time to time to these premises from the works.

In a few cases it will be observed that the disease has come to light in pigs fed on carcases of animals which have died of anthrax. This indicates negligence in notifying disease in the first instance.

The separate heading of recent deaths accounts for a small number of outbreaks, and is important only because it complicates the history of the outbreak. No opportunity was available in these instances to examine material and determine whether the earlier deaths were due to anthrax or to other causes.

The artificial manure of animal origin to which reference is made at the head of the table is in practically all instances a bone preparation. It might be assumed that the process of degreasing and deglueing to which bones are normally subjected in the course of preparation would be sufficient to sterilise the raw material, and in the absence of definite proof that these manures were infected it may well be asked whether their mode of preparation does not warrant their exclusion as a frequent means of introduction of infection.

As examples of evidence that these materials should be regarded in certain cases as a possible means of conveying infection the following cases are quoted:

(*a*) In 7 outbreaks of anthrax which occurred a few years ago bone meal had been used as a manure on each of the farms, and it was found that in every instance the bone meal formed part of one cargo imported from an Eastern country.

(*b*) Bone meal was fed to cows by mistake. Four of these cows became ill and two died of anthrax. The other two, which were also possibly affected with anthrax, recovered.

(*c*) A farmer obtained bone meal for sowing on a crop of barley. A high wind at the time of sowing blew some of the bone meal into the next field, in which two horses were grazing. These two horses died of anthrax, five and eight days respectively after the spreading of the bone meal. These deaths led to legal proceedings, in which the Court found that the horses contracted anthrax from the bone meal, which was said to consist of ground untreated bones.

These instances indicate that preparations of bone may be infected with anthrax. It may be that in certain instances, as in (*c*) above, the bones have not been submitted in the course of manufacture to sterilising processes, or that, although the material

may have been sterilised in the course of manufacture, the general lay-out of the factory has been such that the finished product has been contaminated by contact with raw material. This may occur in cases for example where reversible mechanical transporters are used for the distribution of materials in a factory. In certain cases where all precautions have been taken the sterilised material may have been put in contaminated sacks and thereby have become infected.

It is difficult to assess accurately the part played by artificial feeding stuffs in the spread of anthrax ; a cargo of oilseeds may be divided and sent to various factories, some of which may blend the residuals with others of the same kind or of other kinds, and the feeding stuffs are then distributed throughout the country under different proprietary names, so that individual original consignments are in effect practically untraceable. Occasionally, however, information has been obtained as to the association of one consignment of imported feeding stuffs with a series of outbreaks. For example, six outbreaks of anthrax occurred on farms which had all received a part of a consignment of beans originating from China and ground in this country and distributed to farms as bean meal for feeding to cattle. Another similar consignment was involved in four outbreaks of anthrax on different premises. In certain cases agricultural products originating in heavily infected countries may be contaminated in the country of origin. There are, however, cases in which the crops may have been free from infection when they left the country of origin, but were infected either by materials of animal origin on the vessels on which they are transported to this country or by contaminated sacks. In this connection it may be mentioned that the Ministry of Agriculture and Fisheries has issued a notice to shipowners suggesting that precautions should be taken so that dried hides, wool, hair, etc., should not be carried on top of cargoes of grain or feeding stuffs, and that any hold of a vessel which has carried such animal products should be thoroughly disinfected after the goods have been unloaded. The warning also suggests that sweepings of holds containing grain, etc., should not be mixed with other foodstuffs or be distributed to consignees without informing them of its nature and the danger attaching to using it for feeding animals without sterilisation.

THE USE OF DRUGS IN THE TREATMENT OF DISEASES CAUSED BY WORMS.

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Since 1915 the writer's principal scientific interest has been in the field of anthelmintic medication. In the fifteen years from 1915 to 1930 the investigation of anthelmintics has progressed to the point where it may now be admitted to be actually scientific in approach, method, and technic, and the number of investigators in this field has increased in proportion to an immense increase in interest in the subject. From 1881, the year in which several important treatments for human anchylostomiasis were proposed, following the recognition of the fact that hookworms were serious pathogens responsible for Egyptian chlorosis, tropical anæmia, and the St. Gothard Tunnel disease, to 1915, there had been an increasing interest in the development of anthelmintic medication, but for the most part the studies of this period were along the lines of critical empiricism and increasingly close clinical observation. Previous to 1881 anthelmintic medication was mostly a matter of uncritical empiricism.

In 1915 it was practically impossible to give an intelligent answer to a question in regard to anthelmintic medication in the field of veterinary medicine. Books and journals contained endless formulæ incorporating scores of drugs for the removal of worms from domesticated animals, but there was no critical evidence on which to judge the value of these drugs. The most that was offered was clinical experience, and as clinicians are a mixed lot, comprising good observers and sound thinkers on one hand, and poor observers and unsound thinkers on the other, the recommendations of innumerable John Does and Richard Roes, unknown to the reader, could not be sorted to distinguish the good from the bad.

This situation was extremely uncomfortable for the Zoological Division of the Bureau of Animal Industry, United States Department of Agriculture. A constant stream of questions in correspondence called for answers in keeping with the standing and reputation of the Bureau of Animal Industry. It was only possible to say in reply that John Doe recommended so much ferrous sulphate given thus and so, and Richard Roe recommended so much tartar emetic given this way or that, for the removal of this or that parasite from such and such a host, and this was obviously an unsatisfactory answer. To remedy this situation, the late Dr. B. H. Ransom, a capable and sound scientist, who was then Chief of the Zoological Division, inaugurated a project on anthelmintic medication. The results obtained from that project have paid large returns to agriculture and the veterinary and medical profession, and have done much to stimulate interest in the subject of anthelmintics. The present writer was

detailed in charge of this work, and was associated from time to time with Foster, Shillinger, Cram, and Wigdor. The writer's administrative work of the past five years has prohibited any considerable active participation in these investigations, and the actual investigations at present are being carried out by Dr. W. H. Wright. This paper, therefore, represents the observations of an old campaigner no longer on active duty, and might appropriately be tinged with regret at the necessity for confinement to paper work at a time when the subject of anthelmintics is entering the stage of basic scientific investigation, and when there is active warfare on all fronts and " lovely fighting along the whole line."

Characteristically, the scientist who is no longer a youngster actively engaged in research turns to philosophic speculations, the summarising of knowledge, the correlation of other men's findings, and the formulation of principles. Conforming to this habit, the writer proposes to discuss certain principles of anthelmintic medication. This subject he has discussed in several previous papers, but our principles may be regarded as tentative to the extent that they are subject to modification in the light of additional knowledge, and they need occasional revision. They may be taken as representing ideas which have grown out of an experience that is extensive by comparison with that of other workers in this field, and out of a rapidly increasing fund of available knowledge, and they are of value as background in the consideration of the individual cases the veterinarian will have to consider in practice or research.

Principles of Anthelmintic Medication.

(I) *Factors in Anthelmintic Medication.* Elsewhere the writer has noted that the important factors in anthelmintic medication may be stated alliteratively as : (1) The practitioner ; (2) the patient ; (3) the parasite ; (4) the parasiticide ; (5) the purgative ; and (6) prophylaxis. These may be discussed very briefly as follows :

(1) *The practitioner, i.e.*, the veterinarian or physician, must know all the other factors, not superficially, but well, and must be a person of sound judgment if he is to be trusted with the more or less poisonous drugs which constitute our present armamentarium of anthelmintics. He can be taught, or could be if we had suitable teachers, a sound knowledge of the other factors involved in anthelmintic medication. He cannot be taught to be a person of sound judgment, if he is intrinsically a person of unsound judgment ; the poor thinker will continue to be a dangerous performer with potent drugs, and will continue to discredit excellent drugs by obtaining bad results as a result of bad judgment.

(2) *The patient* must always be considered as to *status præsens* in connection with the other factors, and especially in regard to conditions constituting contraindications for treatment. Failure to consider contraindications is altogether too common, and is a prolific source of fatalities.

(3) *The parasite* must be known definitely, and its habits, location, and life history taken into consideration. A knowledge of these facts leads to sound procedures, and affords an intelligent explanation for certain failures.

(4) *The parasiticide* (the anthelmintic) must be selected not only with reference to its known action on the worms to be removed, but also with reference to its effects on the patient. These drugs are potent and poisonous, they exert a certain amount of unfavourable effect on the patient, and the practitioner must select his drug and his dosage with reference to both of these things.

(5) *The purgative* is usually the most important factor in connection with the procedure of safety. It must be selected with reference to its known action in connection with any anthelmintic, since some purgatives are unsuitable with certain anthelmintics, and either afford inadequate protection to the patient or diminish the efficacy of the anthelmintic. Purgatives should be given in adequate amounts, as small amounts are as bad as, or worse than, none. The time and mode of administering the purgative are also important.

(6) *Prophylaxis* rounds out the job of treatment. The practitioner has not fulfilled his duty to his client until he has not only aided him in his trouble, but also told him why he was in trouble, and how to avoid similar trouble in the future if the state of our knowledge affords such information. The veterinarian who treats a herd of swine in a hot lot, and leaves the swine to pick up millions of ascarid eggs from the ascarids removed and left there, has an inadequate idea as to his duty to his client.

(II) *The Patient as a Factor.* The important thing in connection with the patient is the matter of contraindications (1) for anthelmintic treatment, (2) for certain drugs, or (3) for full doses. These may be briefly stated as follows :

(1) Contraindications for treatment of any sort, as a rule, include extreme weakness, emaciation, and starvation, or febrile conditions, or marked toxic conditions, or extreme youth or age, or pregnancy. Whether these general contraindications hold in a given case is a matter for the practitioner to decide in accordance with his knowledge and judgment. Weak, emaciated, and starved patients will usually benefit by a sound feeding régime to put them in better condition to tolerate treatment, but in cases where weakness and emaciation obviously follow from an insupportable worm burden, good judgment may indicate the administration of diminished doses of an anthelmintic suited to the weakened condition of the patient, with the idea of gradually diminishing the worm burden and enabling the patient to tolerate in time a full therapeutic dose of an anthelmintic.

Febrile conditions, usually associated with bacterial infections, should be treated first and the worms removed after the patient has recovered from the febrile conditions, as a rule ; but the practitioner will have reason to believe, in some cases, that relief from the febrile condition can be more certainly secured by the preliminary removal of part or all of the worm burden. It is impossible to formulate

precise rules on this subject, as every case will be an individual case to be decided by the practitioner.

Marked toxic conditions constitute a contraindication for the additional insult of the more or less poisonous drugs constituting our anthelmintics, as the combined burden may be insupportable. However, there will be times when the practitioner who is well informed as to drug action will elect to use a drug which will impose an injury on an organ not especially affected by the toxic conditions already present, and thus take the worm burden out of the general picture and give the patient this advantage in throwing off the existing toxic condition.

In general, very young or very old animals do not tolerate anthelmintics very well. Very young animals should be surrounded with conditions tending to prevent further infestation and allowed to attain more age resistance before treatment, and very old animals should be regarded as bad risks and allowed to live out their lives with their worms, or be humanely destroyed.

Pregnancy is a general contraindication for anthelmintic treatment, although certain drugs appear to be safe for use in this condition. However, one should try to clear out worm infestations from females before they are bred, not only to avoid any necessity for treatment during pregnancy, but to put the animals in better condition during this critical time, and allow them and the fœtus to benefit accordingly. It is probable that the bad results in the way of abortions and miscarriages in connection with anthelmintic treatments are associated at times with the use of drastic purgatives rather than the action of the anthelmintic.

(2) Contraindications for individual drugs are of many sorts, and follow from the particular action of the drug in question on the host to be treated. In some instances we have alternative drugs having different actions on the host, and one of these may be indicated for use in connection with contraindications for another drug or other drugs. One might say in general that one avoids gastro-intestinal irritants in the presence of gastro-enteritis, chlorinated hydrocarbons causing hepatic necrosis in the presence of serious liver injury, drugs unfavourably affecting the nervous system in the presence of epileptic conditions, etc. All these rules are subject to modification, as we may at times have such results as improvement in cases of inadequate liver functioning after the administration of a drug which injures the liver but stimulates a chronic indolent trouble into an acute condition going on to repair and recovery.

(3) Contraindications for full doses are frequently encountered. In this connection one should consider the apparent age, rather than the chronological age, of an animal, in judging the dose which will be tolerated, and base the dose on size, general condition, and other things. Gross helminthiasis in young animals is a condition which may call for diminished doses and repeated treatments, rather than one full therapeutic dose. Occasionally the killing of large masses of ascarids may cause intestinal obstruction from clumps of worms in

weak animals with an atonic intestine too feeble to remove these masses. The selection of the dose is emphatically a matter calling for sound information and good judgment on the part of the practitioner, and no specific instructions can take the place of such information and judgment.

(III) *The Anthelmintic as a Factor.* The older books define an anthelmintic as a relatively insoluble drug which remains for the most part in the digestive tract, poisons the worms, and then is swept out by a purgative before any considerable amount of absorption can occur, the patient being thus little affected. This defines a somewhat ideal sort of anthelmintic for worms in the digestive tract, but does not represent our present concept of anthelmintics or the actual facts. Anthelmintics at present include drugs used to destroy worms in places outside of the digestive tract, where absorption of the drug from the digestive tract is essential or where the drug is actually given intravenously or in some other manner which precludes the idea that it will not be absorbed and have systemic effects. Moreover, the anthelmintics in use for removing worms from the digestive tract are known to be absorbed to a large extent. Most of the thymol administered and practically all of the carbon tetrachloride in a therapeutic dose are absorbed, although both are relatively insoluble, and carbon tetrachloride is more insoluble in water than is thymol.

In general, anthelmintics are rather selective in their action. One may remove ascarids, but not hookworms or tapeworms; another may remove tapeworms but not nematodes; another may remove hookworms but not ascarids. This fact probably follows in part from the habits, physiology, and structure of the worms. Some anthelmintics have a more restricted range of action than others, but we have as yet no one dependable anthelmintic for all sorts of worms. Such worms as whipworms, situated in the cæcum, and probably rarely subjected to the injurious effects of substances taken by mouth, appear to be highly susceptible to numerous anthelmintics if the drugs come in contact with the worms, and the difficulty here appears to be to find anthelmintics which for one reason or another will get to the cæcum in amounts sufficient to kill these worms.

The form in which an anthelmintic is administered will often affect the result. Thus, fluid extracts may contain active anthelmintics, but the alcohol present may lead to rapid absorption of the anthelmintic in the stomach and upper intestine with the production of systemic effects and the lessening of the anthelmintic effect.

(IV) *Procedures in Anthelmintic Medication.* The procedure in anthelmintic medication may be as important as the selection of the drug. Thus, santonin develops its action against ascarids much more effectively when it is given in small doses repeated daily for a period of time than when it is given in one large dose. Whipworms require the administration of bulky doses of a suitable drug, part of which is likely to enter the cecum, or else the repeated administration of small doses of a suitable, non-irritant drug over a period of time in order to increase the likelihood that some part of these doses will enter the

cæcum. Preliminary purgation, preferably with salts, appears to be important in treatments for the removal of tapeworms, presumably because it is necessary to clear the mucus away from the head of the worm if complete removal is to be obtained. The use of the duodenal tube has been recommended for the administration of anthelmintics for the removal of tapeworms in order to avoid gastric absorption of the drug, a point which deserves further study.

Fasting is a matter of importance. It is not true, as was at one time supposed, that fasting starves the worms and leads them to ingest an anthelmintic carried in a tempting vehicle, such as milk, as we know that ascarids may be kept alive for 26 days in such an innutritious medium as Kronecker's solution. However, fasting is important in preventing the dilution of the drug in food masses, in preventing the escape of worms protected from the anthelmintic by food masses, and in preventing the bad effects often noted when anthelmintics are administered on a full stomach with the digestive processes active. Our reasons for fasting are largely theoretical, but practice supports the general contention, and experimental evidence shows definitely that anthelmintics administered in food are usually ineffective or require administration over long periods to compare in efficacy with drugs given in therapeutic doses to fasting animals.

The length of the preliminary fast necessary for effective anthelmintic action varies with different animals. In the cases of man and the dog and cat, fasting overnight appears to be sufficient and better than a longer period. In these animals the simple stomach empties rather promptly, and prolonged fasting puts the patient in an abnormal condition in which the patient's discomfort and other bad effects of the anthelmintic are increased. Swine should be fasted at least 24 hours. Horses should be fasted 12 to 24 hours before treatments for stomach worms, bots, and ascarids, but fasted 36 hours before treatments for strongyles in the large intestine. It is hopeless to try to empty the rumen of ruminants, and the treatments which have been found effective against worms in these animals are usually effective after an overnight fast or regardless of fasting. An interesting development in this connection is the finding of Minot and Cutler that carbon tetrachloride may induce guanidine retention, and as a practical point it might be noted that quite generally deaths in sheep from the small amount of carbon tetrachloride, 1 c.c., used in treatments for liver flukes, have followed in the United States only in cases where sheep were on feed other than pasture grass alone, and not where they were on pasture. As a practical measure for avoiding these deaths, Dr. Robert Jay, of the Zoological Division, who is in charge of liver-fluke-control work in California, has advocated putting sheep back on a low-maintenance diet on pasture for a few days before treating with carbon tetrachloride.

After treatment, in the cases of man, dogs, and cats, food should be withheld for three or four hours, or until the bowels move. Swine should not be fed until after a longer period, perhaps six hours. Information appears to be lacking as to the correct procedure for

horses and ruminants, but at this time we are inclined to think that feed should be withheld for three or four hours after treatment. This may not be necessary with ruminants. With most animals the drug should be given time to leave the stomach before food is allowed.

V. *Purgation.* Purgation is protective; in fact, it is the most important of the routine procedures in connection with the safety and comfort of the patient. In the treatment of human patients, from whom we can best obtain information as to certain symptoms caused by anthelmintic medication, practically all the anthelmintics administered by mouth produce, in a large proportion of cases, headache, dizziness, or nausea, or any two or all of these symptoms, the nausea being associated both with the anthelmintic and the purgative. These symptoms quite generally abate or disappear when the bowels move. In human medicine it appears to be advisable to follow this procedure: The patient should report to the physician or nurse four hours after treatment, and if the bowels have not moved, another dose of a fast-acting purgative, such as salts, should be given, and the patient told to report again in four hours; if the bowels have not moved eight hours after treatment, purgatives, enemas, and other measures should be used to obtain purgation. Failure to obtain purgation may lead to serious consequences.

In veterinary medicine no standardised procedures have been developed in connection with ensuring purgation, but the veterinarian should keep in mind that purgation is highly important, and take the necessary steps to ensure it. Such drugs as arecoline hydrobromide hypodermically may be of value in cases of delayed purgation. In this connection, it may be noted that ordinarily it is unnecessary to resort to preliminary purgation before anthelmintic treatment. There are, however, certain exceptions. Patients which are constipated should be given a purgative the night before treatment; otherwise, it may be difficult to secure purgation promptly after treatment. Preliminary purgation is also necessary or desirable in connection with treatments for tapeworm infestations, as the use of such drugs as salts apparently serves to clear the mucus away from the tapeworm head and thus expose it to the action of the anthelmintic.

The choice of a purgative and the procedure in administering it must be based on the ascertained action of a given purgative in connection with a given anthelmintic. Experimental evidence indicates that castor oil is very satisfactory when given simultaneously with chenopodium in ascariasis, the chenopodium displaying a high efficacy and the castor oil being highly protective. Critical evidence indicates that castor oil apparently does not increase the absorption of such drugs as chenopodium and male fern, and that in adequate doses it will protect patients against the effects of even lethal doses of these drugs. On the other hand, castor oil lowers the efficacy of carbon tetrachloride against hookworms. Why purgatives protect the patient as much as they do is not clear. It does not appear to be true that they sweep from the digestive tract any considerable

amount of unabsorbed drug. Theoretically, what they do is to distribute the insult of the anthelmintic over a larger area of mucosa and over a longer time period by carrying the anthelmintic farther along the digestive tract and retarding absorption. Castor oil should be used, as a rule, only in those cases in which it can be given with the anthelmintic. If the purgative is to be given an hour or two after the anthelmintic, it is advisable to use the fast-acting purgatives, such as salts.

Macht has shown that salts, by virtue of the well-known salt action, lessen or inhibit the absorption of drugs given with them. They are, therefore, contraindicated for use with drugs from which a systemic effect is desired. On the other hand, the salt action is of value in connection with anthelmintics for removing worms from the digestive tract proper, since systemic action is not desired from these drugs, and it is desired to confine their action to one exerted within the lumen of the digestive canal. In using salts, the present tendency is to substitute sodium sulphate for magnesium sulphate on the ground that sodium is less toxic than magnesium, and does not have the same antagonistic action with the blood calcium. Experience in human medicine shows that saturated solutions of salts produce more nausea and other bad effects than do weaker solutions, and it is advisable to make a saturated solution and dilute it with twice its bulk of water for use. Salts are most readily used in veterinary medicine in patients which do not vomit, or vomit with difficulty, such as ruminants and solipeds. Dogs show a disposition to vomit after salts, although this tendency can be overcome, and salts can be used in dog practice.

Liquid petrolatum in some form is often used in connection with anthelmintics, and may be satisfactory in the treatment of such animals as the horse. Tests show that it is unsatisfactory in combination with chenopodium in the treatment of dogs, as it lowers the anthelmintic efficacy and gives inadequate protection against the effects of the drug on the dog. A more extended study should be made as to the effect of varying the amount of petrolatum, as this may be a factor in determining the results.

Olive oil is sometimes used in connection with anthelmintics, but experience indicates that this is usually bad practice. Olive oil is one of the substances which remain longest in the stomach, and in combination with chenopodium there results a decided inflammation of the stomach from the prolonged irritant action of the chenopodium at this point. There is also a resultant fall in efficacy due to the absorption of the drug in the stomach.

In veterinary medicine the value of our anthelmintics must be ascertained by critical testing if we are to have dependable information. In critical testing, animals are given measured doses of the drugs to be tested, all worms passed for a suitable period are collected, identified, and counted, and the animals are then killed, and all worms present post-mortem are collected, identified, and counted. These two sets of figures give the total numbers of worms of each kind

present at the beginning of the experiment, and one can compute rather accurately the percentage of efficacy of the drug, provided a sufficiently large number of experiment animals is used.

This method of ascertaining efficacy by critical testing has its limitations. Worms destroyed in the stomach usually undergo complete digestion, or are partially digested before passing to the intestine. Further, this method cannot be depended on to ascertain contraindications for the use of anthelmintics owing to the limitation in the number of animals which can be used. One may ascertain much as to toxicity of the drug by using high doses, and one can find some test animals with such general contraindications as febrile conditions, extreme youth, etc., but it requires the larger test of clinical experience in various places and at various times to ascertain possible contraindications of all sorts. Here is a field of observation to which insufficient attention is paid by the veterinarian in practice. He keeps too few clinical records, and spends too little time in their analysis, to furnish the information he might furnish along this line. For this reason the pharmocologist would have to be depended on for the most part, to ascertain the effect of a drug on an animal in order to give us a general basis on which to predicate contraindications.

The alternative to critical testing is investigation by the egg-count method, and this method has decided limitations. Egg-counts do not show male worms, larval or immature worms, sterile females, or females in which egg production has been inhibited temporarily by drug action. Their value is also limited by our limited knowledge or ignorance of egg ratios for many worms, our inability to distinguish the eggs of many of the various worm species, variations in egg production and in character and amount of the fæces, and errors in technic.

In clinical experience combined with laboratory procedures for checking anthelmintic treatments, there are certain things which may be kept in mind in connection with the appearance of eggs in the fæces after treatment. These eggs may indicate what they seem to indicate—the complete or partial failure of the anthelmintic. On the other hand, they may indicate the appearance in the digestive tract of worms which had been wandering through the tissues or developing in cysts at the time the treatment was given, especially if the fæces are examined several days after treatment. They may also indicate that worms were inaccessible at the time of treatment, as would be the case with egg-producing female ascarids in the bile ducts.

In judging the value of an anthelmintic, one must allow time for the passage of the dead worms. Dog ascarids may pass for seven days, although over 80 per cent. come away the first 24 hours. An examination of the fæces of dogs for four days will account for 98 per cent. of the worms passed. Horses may require 17 days or longer to pass all of the bots destroyed by a drug, as the bots may wander around in the cæcum for a long time before leaving it.

Our failure to remove worms may be explained in various ways, and there are some failures for which we have no explanation. In some cases we have no known anthelmintic with the selective action necessary for the removal of the worms. In general, the larger worms in the digestive tract are more readily removable than the smaller, a thing which Railliet and the writer have both noted previously. The writer would be inclined to think that the greater size is actually a reason why larger worms are more readily removed, since a worm with a diameter relatively large in comparison with the size of the lumen of the tube in which it lives is more certain to have at least part of the body projecting out of the mucus and other contents of the tube, and hence accessible to anthelmintic attack. The difficulty in removing the smaller worms appears to be associated with the fact that their small diameter permits them to lie in contact with the mucosa with a layer of mucus and other material above them which mechanically prevents anthelmintics from coming in contact with them ; or these small worms may be actually embedded in the mucosa, or buried in nodules out of reach of drugs passing through the lumen of the digestive canal. Similarly, larger worms, such as ascarids, may be inaccessible to anthelmintics if they are in the bile ducts or pancreatic ducts.

Partial failures follow in part from the fact that we have no known drugs and developed methods of procedure which result in 100 per cent. removal of worms in 100 per cent. of animals treated, for any species of worm or host animal. Drugs which remove on an average over large series of cases from 90 to 100 per cent. of any kind of worms may be regarded as excellent. In default of such drugs we must use the best we have until we can develop better drugs.

PYOBACILLOSIS IN SHEEP.

By Walter Jowett, f.r.c.v.s., d.v.h.,

Edinburgh.

The object of this note is to give a brief account of investigations which were conducted by the writer on material—mainly the lungs and related lymphatic glands of diseased sheep—obtained from the Edinburgh Abattoir during the past year. At the outset the investigation was initiated with the object of ascertaining whether or not the disease caseous lymphadenitis occurred amongst local sheep. As is, of course, well known, this disease is of considerable economic importance, and of anything but rare occurrence in some of the countries which export mutton carcases to Great Britain. Having encountered it in South Africa, the writer was acquainted with its characteristics, and this experience proved of some advantage in the course of the investigation.

Although some of the lesions seen in local sheep perhaps bore a superficial resemblance to the disease named, yet when bacteriological tests were carried out there was never any difficulty in disproving their analogy to caseous lymphadentitis, and so that disease has not been encountered by the present writer in any of the locally slaughtered sheep so far examined.

In every case cultures were made from the pus present in the foci and in several instances biological tests were carried out in addition. The cultures yielded a variety of organisms, amongst which may be mentioned the ordinary pyococci, streptococci, and occasionally the B. necrosis. In several instances a pyobacillosis due to a Gram-positive bacillus was encountered, and also another infection due to a Gram-negative organism. In this account it is proposed to give a short description of the former, reserving a description of the last mentioned pyogenic infection (i.e., the one due to the Gram-negative organism) until a later date.

The disease now under consideration was first brought to the writer's notice during the late summer of 1929, when Mr. E. J. H. Sewell, m.r.c.v.s., having then observed amongst the sheep arriving at the Edinburgh Abattoir a number which, although in apparently good condition, showed pronounced symptoms of distressed breathing, obtained and forwarded lungs, etc. from these animals for investigation with a view to ascertaining the nature of the diseased condition. According to the particulars furnished, all the subjects were at least six months old, several over that age. Many of them had been dipped recently in an arsenical dip, and as in some cases double dipping had been carried out at short intervals it was at first thought this might possibly have had some connection with the disease. Such indeed might conceivably be the case, at any rate in so far as the dipping might possibly have had an influence

in lowering resistance to infection, or perhaps in the same connection favoured the extension of a diseased process already in existence. There was, however, no evidence of poisoning from the dipping, or of traumatic pneumonia ; moreover, it was stated that a chemical analysis had already been made with entirely negative results.

Briefly, the facts are that on slaughter there was usually found well marked broncho-pneumonia and pleuro-pneumonia, with in several cases a considerable quantity of fluid in the pleural cavity. This latter was in some cases clear, straw-coloured, and odourless, but in others—these being the more advanced and more numerous cases and those which showed the most marked signs of illness (distressed breathing, etc.) during life—the fluid was turbid, contained flakes of lymph and pus, and emitted a distinctly objectionable odour. One was evidently dealing here with a septic pleurisy. The amount of fluid present in these cases was, as already mentioned, often very considerable, but, unfortunately, accurate measurements were not recorded. The objectionable odour was evanescent, disappearing in the course of two or three hours or so after exposure to the air.

On examining the lungs the consolidated or hepatised area was generally, although not invariably, the anterior portion, and it was in some cases covered by a layer of coagulated lymph, often an inch or more in thickness. An outstanding feature was the sharp line of demarcation separating the diseased from the still healthy portion of lung. In the involved area the interlobular septae were not infrequently found thickened and infiltrated with lymph, sometimes markedly so. In several cases, on incising the consolidated portion of lung, one or more abscesses containing thick creamy pus of a greenish or greyish-green colour were found, this pus in apparently later stages being thickened and almost caseous in consistence. *The related lymphatic glands did not usually present any marked alteration beyond some increase in size,* in many cases even this was not apparent.

In a few cases one of the lungs was found adherent to the chest wall, whilst in others the lungs contained merely encapsulated abscesses, the contents being a greenish or greenish-grey pasty pus with an objectionable odour. In these last mentioned cases only small areas of lung were found consolidated. These were regarded as cases in process of recovery.

In some evidently chronic cases an area or areas of one or both lungs showed distinctly fibroid changes and on section of such areas purulent foci were disclosed, the pus being similar to that described above. One outstanding case showed a necrotic mass bathed in evil smelling pus in the substance of one lung and both lungs showed pleuritic adhesions, mainly at the apices. In yet another striking case, in addition to abscesses in the substance of both lungs, an abscess the size of a walnut was found on the surface of the heart (sub-epicardial, near the base of the left ven-

tricle). This case, unfortunately, was not examined ante-mortem and it is therefore impossible to record whether any marked symptoms of illness or distress were evident.

Many of these cases in which chronic lesions were found in the lungs (discrete abscesses, etc.) showed no obvious symptoms of ill health prior to slaughter—a fact which has occasioned considerable surprise ; it has not been an unusual experience to find quite large pulmonary abscesses in well conditioned animals which manifested no appreciable signs of ill health immediately prior to slaughter. These, of course, were chronic cases, or those in process of recovery. Possibly in the earlier and more acute stages signs of respiratory disturbance would have been observed had the subjects been specially examined.

In those cases presenting lesions of lung consolidation alone or complicated with clear straw-coloured exudate in the pleural cavity, the *Bacterium ovisepticum* was obtained in culture from the involved area. On isolation and testing this organism on experimental animals, however, it was found to be of low virulence. In the more numerous instances, in which the abscesses mentioned were a complicating factor, as also in those in which an evil smelling pleural exudate was a feature, one was able to demonstrate the presence of a Gram-positive bacillus, either in pure culture or together with the ovoid bacterium (pasteurella) or in the more chronic cases with a streptococcus or occasionally with the *B. necrosis*. This Gram-positive bacillus resembled very closely in its morphological appearance the *Corynebacterium pyogenes*, but showed certain differences, more especially in its degree of pathogenicity for experimental animals and also in its effect on certain culture media, being, for example, decidedly less markedly proteolytic in its action on solidified blood serum. Otherwise it conforms

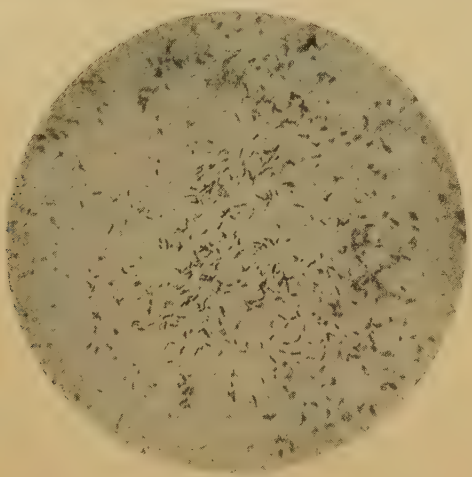

FIG. 1.
Film preparation from pleural exudate of a sheep. Stained by Gram's method. × 1000.

to most of the known characteristics of the *Corynebacterium pyogenes* as described elsewhere*.

The main characteristics of the organism isolated from the above described lesions in sheep are as follows :—

When examined in films from pus, etc., the organism appears as a small rod and also in coccoid forms, being markedly pleomorphic. In such preparations the bacillary forms are usually well in evidence, but when cultivated on artificial media either coccoid or bacillary forms may predominate almost exclusively, or the two forms may be more or less evenly distributed throughout the film.

Diplococcal forms are at times encountered in culture and sometimes numerous, but when such forms are transplanted to different culture media the rod form re-appears. Occasionally the organism, when in the bacillary form, may appear somewhat swollen at one of its ends or in the centre. Small distinctly formed clubs are sometimes seen in films from cultures, more especially when prepared from Lœffler's serum cultures. The organism stains well

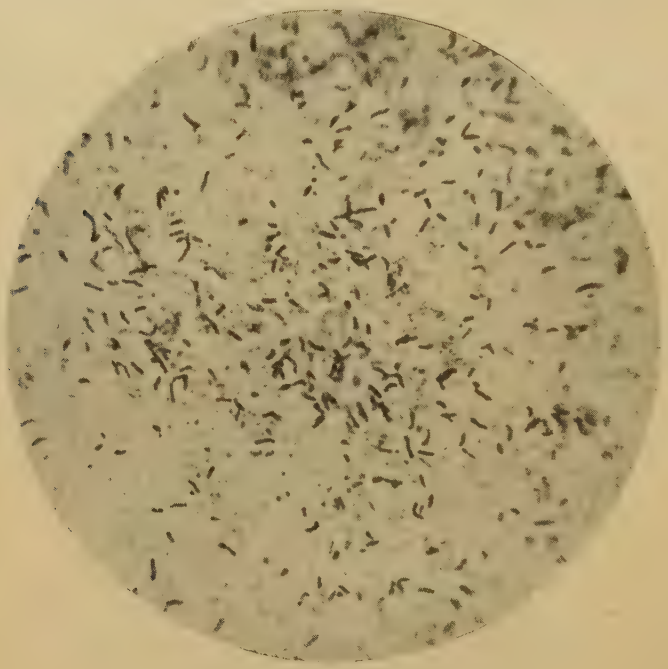

Fig 2.
Same as Fig. 1, more highly magnified.

by the Gram method and the staining is usually uniform and regular, at any rate in films prepared from pus and from young cultures. In older cultures—perhaps more often in preparations from Lœffler's serum cultures than others—irregular staining may be observed,

* Journal of Comparative Pathology and Therapeutics. Vol. 38, p. 180, 1925.

in which event the organism assumes a somewhat granular appearance. In cultures, usually only after several days' incubation, a percentage of the organisms present tend to take the contrast stain, wholly or partially, but notwithstanding this the organism should be classed as distinctly Gram-positive. Spores are not evident and a capsule could not be demonstrated.

The organism is an aerobe and a facultative anaerobe. The optimum temperature is about 37° C. There is little or no growth at room temperature. To maintain the cultures transplantation every two or three weeks is necessary.

On ordinary plain agar the growth is negligible, the addition of blood or serum or other body fluids being apparently necessary for growth to take place. Milk forms a good medium and in this fluid the organism shows distinct bacillary forms.

In litmus milk the growth is practically identical with that of the bovine strains of *C. pyogenes*, producing acidity and clotting. The appearance presented by the culture in this medium in the course of twenty-four to forty-eight hours after inoculation is that of a pink to white sedimented mass with a clear pink or red supernatant fluid. Later the clot undergoes digestion. The medium remains acid.

On Lœffler's serum the organism grows readily but generally without showing pronounced liquefying action on the medium, this, as already mentioned, being in contrast with strains of *C. pyogenes* of bovine origin, in which "pitting" and liquefaction is a well marked feature. In the case of one of the strains (ovine) worked with, after passage through a rabbit the liquefying action on serum showed some increase after recovery from the rabbit, although still decidedly less than occurs with strains from bovine sources.

In blood bouillon tubes (with bovine red blood corpuscles) haemolysis was rapid and complete, more rapid, in fact, than was the case with a bovine strain of *C. pyogenes* tested at the same time and in a corresponding manner as a control. On blood agar plates haemolysis was perhaps less marked although still well in evidence, especially after repeated subcultivation on blood agar.

On blood gelatine and serum gelatine at 24° C. liquefaction of the medium was evident in the area in which growth occurred.

In serum bouillon growth occured readily, and when examined after 48 hours' incubation many coccoid forms together with a few short chains in the form of diplococci and diplostreptococci were seen in films prepared from the culture.

Fermentation Reactions.

The medium utilised for testing the action of the organism on various sugars was a bullock's heart bouillon, to which ten per cent. sheep serum was added, the test substance (0.8 per cent.) and the indicator (Andrade's or Kubel-Tiemann Litmus solution) being added prior to the final sterilisation.

The following table shows the results obtained with the organism in question :

Glucose.	*Lactose.*	*Saccharose.*	*Mannite.*	*Maltose.*	*Dulcite.*	*Adonite.*	*Salicin.*	*Litmus Milk.*
A. (2)	A. (6)	—	—	A. (2)	—	—	—	A.C.D. (A.C. 4)

For the purpose of comparison a strain of *C. pyogenes* (*bovis*), recently isolated from lesions (multiple abscesses) in the lungs of a calf, was tested in the same manner. The results were as follows :

Glucose.	*Lactose.*	*Saccharose.*	*Mannite.*	*Maltose.*	*Dulcite.*	*Adonite.*	*Salicin.*	*Litmus Milk.*
A. (2)	A. (2)	—	—	A. (12)	—	—	—	A.C.D. (A.C. 2)

In the same connection a corresponding test was put up with a strain of the Priesz-Nocard bacillus (*C. pseudo-tuberculosis ovis*), isolated from a caseous lymph gland in an imported mutton carcase. The following were the results :

Glucose.	*Lactose.*	*Saccharose.*	*Mannite.*	*Maltose.*	*Dulcite.*	*Adonite.*	*Salicin.*	*Litmus Milk.*
A. (14)	—	—	—	—	—	—	—	No change.

A. = Acid formation. The figures against the letters indicate the period when first observed, thus A. (2) = acid evident on second day's incubation. Gas was not formed in any instance.
A.C. = Acid production and clotting, with
D. = Subsequent digestion of the clot.
(Observation was prolonged over a period of three weeks.)

The above fermentation tests indicate the close relationship in effects produced in the sugar media of the organism under consideration and the *C. pyogenes* (*bovis*), whilst differentiating both these organisms from the *C. pseudo-tuberculosis ovis* (Priesz-Nocard bacillus). Especially striking is the effect produced in litmus milk, for while both the *C. pyogenes* (*bovis*) and the organism now under discussion give rise to acid clotting with subsequent digestion of the clot, the effect is totally different in the case of the Priesz-Nocard bacillus, with which no change is produced in litmus milk.

Agglutination Tests.

The different strains obtained from sheep were submitted to the agglutination test, serum obtained from an inoculated rabbit being

utilised for the purpose. The different strains tested were found to be identical. Serum from a rabbit which had been immunised against *C. pyogenes* (*bovis*) was also tested on the sheep strains, and produced results which further tended to indicate the identity of the latter with the former. Considerable difficulty was experienced in the tests owing to the occurrence of spontaneous agglutination, which was not fully obviated by varying the percentage of the saline content of the diluent, and the phenomenon referred to was manifested in recently isolated strains as well as in those which had been maintained for a time in the laboratory.

Agglutination absorption tests were also carried out, using serum from a rabbit which had been immunised against bovine strains of *C. pyogenes* and testing after absorption both on the homologous and the sheep strains. The results again, owing to the occurrence of spontaneous agglutination, were not " clear cut," but they tended, nevertheless, to indicate the identity of the organism now described with the *Corynebacterium pyogenes* (*bovis*).

Pathogenicity.

The virulence of the different strains obtained from sheep was tested on guinea-pigs and rabbits.

It was found that the degree of virulence of the strains dealt with showed a distinct variation in accordance, apparently, with the source of origin, *i.e.*, those from the acute cases complicated with pleurisy, etc., were decidedly more pathogenic than strains isolated from the more chronic cases with encapsulated abscesses. In general, the organism showed itself more pathogenic for guinea-pigs than for rabbits, this being, of course, in direct conflict with *C. pyogenes* (bovis et suis), in which the position is the reverse. One strain in particular, isolated from a sheep showing acute symptoms of pneumonia with pleurisy, proved of special interest. When first isolated this organism was devoid of pathogenic effect on the rabbit. By passage through guinea-pigs (direct from guinea-pig to guinea-pig by intraperitoneal inoculation) this strain became exalted in virulence, and finally proved fatal when inoculated intraperitoneally into a rabbit. These results are at variance with those of Descazeaux,* who, working in Chili with an apparently similar or related organism, found no exaltation of virulence by passage through experimental animals, although this observer records that passage through young lambs and sheep did decidedly exalt the virulence of the organism with which he worked.

Strains isolated from the more chronic lesions, *i.e.*, those encountered in older sheep in good condition and not having shown obvious symptoms of illness prior to slaughter, and in which the lesions consisted merely of encapsulated abscesses in the lungs, produced only local abscesses at the point of inoculation, the inoculated animals showing no other marked ill effects.

* Descazeaux. Rev. Gén. de Méd. Véterinaire. T. 37, p. 193, 1928.

Finally, it may be mentioned that in no instance was evidence of Strauss's reaction seen when any of the strains were inoculated intraperitoneally into male guinea-pigs, whereas with the Priesz-Nocard bacillus this reaction was readily produced.

Summary.

An organism encountered in certain lesions in sheep bears a close morphological resemblance to, but is distinct from, the *Corynebacterium pyogenes* (bovis et suis).

The organism in question is considered to be the *C. pyogenes* (*Bacillus pyogenes*), which has apparently become adapted to the sheep, in passage through which animal it has undergone certain modifications.

The following is a summary of experiments conducted with one of the strains isolated from sheep. These support certain of the foregoing statements:

Guinea-Pigs.

Guinea-pig A.—March 28th, 1930.—Inoculated intraperitoneally suspension in saline of about one platinum loopful culture. Strain A (isolated from sheep's lung about six months previously and since maintained in the laboratory). This strain had not passed through a guinea-pig previously.

Result.—Died seventh day after inoculation. Septic (purulent) peritonitis. Turbid blood-stained fluid in peritoneal cavity with flakes of pus on organs and adhesions. Organism recovered in pure culture from peritoneal exudate and also from heart's blood.

Guinea-pig B.—March 4th, 1930. Inoculated intraperitoneally about 0·5 c.c. peritoneal exudate of G.P.A. (see above).

Result.—Died three days after inoculation. P.M. findings similar to above. Organism recovered in pure culture from peritoneal exudate and heart's blood.

Guinea-pig C.—March 4th, 1930. Inoculated subcutaneously about 1 c.c. peritoneal exudate G.P.A.

Result.—Large swelling and abscess formation at site of inoculation. Killed 13 days after inoculation. Organism recovered in pure culture from pus.

Rabbits.

Rabbit No. 1.—August 10th, 1929.—Inoculated intravenously 0·5 c.c. suspension in saline growth from 48 hours culture (serum agar) of organism isolated from lesions lung of sheep. September 9th, 1929.—Re-inoculated about two-thirds growth culture, as above, intraperitoneally. September 30th, 1929.—Re-inoculated same, also subcutaneously, one-third culture. October 13th, 1929.—Blood withdrawn for testing and animal again re-inoculated as above. October 20th, 1929.—Blood withdrawn for testing, and also at later date.

Result.—Animal killed February 11th, 1930. Normal, no abscesses present. Had increased in weight.

Rabbit No. 2.—Inoculated intraperitoneally three platinum loopfuls from growth on 48 hours culture (blood agar). Strain " A " (isolated originally from sheep and since passed through guinea-pig A).

Result.—Died 18 days after inoculation. Purulent peritonitis. All abdominal organs congested. Adhesions. Considerable amount of pus present, especially in region of liver. Organism recovered in pure culture from pus.

Second Series of Experiments illustrating the exaltation of virulence as result of passage.

Guinea-pig 1.—Inoculated intraperitoneally about 0·5 c.c. suspension in saline of organism recently isolated from lesions in lungs of sheep. Suspension prepared from 48 hours serum agar culture. Male guinea-pig.

Result.—Abscess at site of inoculation. No evidence of Strauss's reaction. No marked ill effects. Killed about six weeks after inoculation. Organism recovered from pus in abscess. No other lesions.

Guinea-pig 2.—March 17th, 1930. Inoculated intraperitoneally suspension from culture organism isolated from abscess Guinea-pig 1, four months previously and since maintained in the laboratory on blood agar.

Result.—Died four days after inoculation. Purulent peritonitis. Organism recovered from purulent exudate in peritoneum. No growth from heart's blood.

Guinea-pig 3.—March 24th, 1930. Inoculated subcutaneously same material as used for inoculation G.P. 2 (above).

Result.—Abscess formation, otherwise no effect. Killed three weeks later. Organism recovered.

Guinea-pig 4.—April 1st, 1930. Inoculated intraperitoneally loopful culture isolated from G.P. 2.

Result.—Ill, wasting. Died seven days after inoculation. Organism recovered in pure culture from peritoneal exudate, also from heart's blood.

Guinea-pig 5.—April 8th, 1930. Inoculated subcutaneously peritoneal exudate G.P.4 direct. About 1 c.c.

Result.—Obviously very ill day following inoculation. Died sixth day. Creamy pus at site of inoculation. All subcutaneous tissue thoracic and abdominal region congested and presented a sodden appearance.

Guinea-pig 6.—Inoculated intraperitoneally 0·5 c.c. peritoneal exudate G.P. 4 direct.

Result.—Died about 54 hours after inoculation. Obviously very ill day following inoculation. Purulent peritonitis. Sanguineous fluid in peritoneum, flakes of lymph and pus on surface of viscera. Organism recovered in pure culture.

Guinea-pig 7.—Inoculated subcutaneously peritoneal exudate G.P. 6. Direct (about 1 c.c.). Died three days after inoculation. Creamy pus at site of inoculation. Organism recovered from pus.

AN UNUSUAL STRAIN OF BRUCELLA CAUSING ABORTION OF CATTLE IN PALESTINE.

By S. J. Gilbert, m.r.c.v.s.,
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The possibility of negative results being obtained in applying the agglutination test to the blood of aborting cattle unless various strains of the Br. abortus are used, has been pointed out by Good, Dimoch, and Harms[1], and also by Traum and Henry[2]. Definite groups have also been described by other authors. The strain which is the subject of this article presented on a closer examination such peculiar features as to be worthy of attention, especially in view of the increasing importance of the relationship of the Br. abortus to Undulant Fever of human beings.

A few years ago agglutination tests in connection with the control of contagious bovine abortion were commenced by us. The strain first used was obtained from the Lister Institute (No. E.624) and has been in regular use ever since. Subsequently three other strains, one of which was serologically allied to Br. melitensis, were received from India, through the kindness of the Director of the Imperial Institute of Veterinary Research.

The history of abortion in Palestine is somewhat peculiar. During the first two years under the Mandate clinical abortion in cattle appeared to be non-existent or at least to occur so rarely as to pass all notice, with the exception of one virulent outbreak in a herd of cattle in Jerusalem. These cattle had either been imported from Damascus or were bred from that type on the premises. The owner of the herd subsequently disposed of them by slaughter and raised a fresh herd in adjacent buildings. This new herd has since remained free from the disease. Tests of the infected cattle were performed by the Central Laboratory, Department of Public Health, and were found positive for Br. abortus. The origin of the outbreak could not be traced.

Soon afterwards the blood of native cattle, taken in different parts of the country, was tested at the above-mentioned Laboratory, with a result of 30% positive[3]. No abortions however could be traced in any of these localities.

To meet the urgent demand for improved dairy cattle, large numbers of European cows, particularly from Holland, were imported during the subsequent two years and distributed to various centres. Such cows had mostly been tested prior to export. During the following year abortions commenced to occur in some of these centres and in a few instances could be directly traced to the imported cattle. Although material has been very difficult to obtain under local conditions, the organism has been isolated from three affected centres and found by absorption tests to be identical with the European strain employed in our routine tests.

After some time, however, it became noticeable that a considerable number of actual abortions occurring in various places were proving negative. Current tick fevers and other conditions were in some cases undoubtedly responsible for these, but the percentage of negative results to the test still appeared to be rather high. We have had ample opportunity of checking our results, especially in those cases where the type of organism responsible is known, and we have found the agglutination test as accurate in its application as has been affirmed by McFadyean[4], at least as far as typical European strains are concerned. At the same time, the possibility of other and serologically different strains could not be overlooked. The first indication that this was an active factor came to light when cattle from the old established settlement of Petach-Tikvah were being tested. The majority of these cattle are of a rather poor native type with some admixture of imported blood.

Some indefinite reactions having occurred, the test was made against the " aborto-melitensis " strain received from India. With an emulsion from this strain reactions were more definite, and some previously negative results were positive though none were of very high titre.

A fresh foetus was obtained from an aborting cow which had recently been brought to Jaffa from this settlement. Isolation of an organism from the stomach contents of this foetus was successful, but the organism did not grow under normal aerobic conditions until the 8th sub-culture. The method adopted for isolation was to obtain a low oxygen content in a sealed jar which contained the culture and a small lighted spirit lamp.

As all abortions which occur are not tested, it is difficult to estimate the percentage of actually aborting cows which prove negative. On testing sera from such cows, however, against E.624 and the new or local strain, the percentage which previously proved negative was greatly reduced. 20 tests of a number of aborting cows over a period of a year were either positive only to local strain or more strongly positive to that strain. These cases occurred as occasional abortions and were not followed by anything in the nature of an outbreak.

The Local Strain.

Straining reactions are similar to those of other strains, as is also the microscopic appearance, but if anything shorter forms appear to be more common. Tests of a number of sugars were identical with the rest of the group in being of a negative character. The recent work of Traum and Henry[2] on the different appearances of colonies plated from antigens which did not agglutinate well, led to a closer examination being made of individual colonies. On agar plates at least two distinct colonies were observed, viz. :— (1) lenticular and (2) round.

The former had a well defined outline andappea red more compact. The latter showed a slightly undulating margin and were less opaque; both were finely granular. Better emulsions were obtained from the

lenticular colonies, but further work is being carried out in regard to this matter.

Comparison of Strains.

The following strains were used for comparison :—

1.—English (E.624).

2.—Br. Melitensis—An undulant fever strain also obtained from the Lister Institute.

Growth on Potato.

The growth of the local strain on potato took place more slowly and was less in quantity than that of the other types.

1. *Local.* Honey-coloured but somewhat paler than Melitensis. Remained unchanged in colour.

2. *English.* Honey-coloured, becoming brownish after one week and a chocolate colour after a few weeks.

3. *Melitensis.* Honey-coloured. Remained unchanged.

The "aborto-melitensis" strain received from India was also grown on potato and did not assume the dark chocolate colour seen in typical strains of Br. abortus. Organisms isolated by us from acute outbreaks of abortion have shown this characteristic colour when grown on potato.

Lead Acetate Test.

Very clear-cut results were obtained by growth on Stafseth's liver agar, incubated for 48 hours. Liberation of hydrogen sulphide was marked with both the English and Local strains. The Melitensis culture was negative.

Pathogenicity.

Pregnant guinea-pigs were inoculated subcutaneously with 0.3 cc. of a heavy emulsion of English, Local, and Melitensis strains. All failed to abort and remained clinically unaffected. The Local strain, however, had now been sub-cultured for a period of more than 18 months and the other strains for some years.

Tests of Native Cattle.

A previous test of blood from cattle taken at the Jaffa Slaughter-House showed 12% positive to English strain and 5% to the Local. As it was possible that cattle of a mixed breed which had come from infected colonies had been included, a further test was carried out on 29 native cattle from a village near Gaza, where there was very little likelihood of imported blood having been introduced. The results, shown as follows, were very different :—

	1	1	1	*Higher*	*Negative*	*Total*
	25	50	100			
English Strain	–	–	–	1 (1 : 800)	28	29
Local Strain	7	1	1	1 (1 : 400)	19	29

The cow showing a reaction at 1 : 800 was also positive to Local strain up to 1 : 400 as shown above. Subsequent enquiry showed that this animal had been purchased in an open market a few years ago. No abortions had been known to occur.

Tests of Native Goats.

Sera from 133 native goats were tested against the Local strain, and 75 of these gave positive results in titres from 1 : 25 up to 1 : 100 and three were positive in a dilution of 1 : 200—a total of 56%.

Tests of goats have repeatedly been carried out with emulsions. of Br. melitensis with an average positive result of 5 to 6%

For purposes of comparison the sera of fifteen native goats were tested against the three strains, the positive results being as follows :—(Four tubes were used in each test, viz., 1 : 25, 1 : 50, 1 : 100, and 1 : 200).

	English	*Local*	*Melitensis*
1	- - - -	† † † -	- - - -
2	- - - -	† † † -	- - - -
3	- - - -	- - - -	- - - -
4	- - - -	- - - -	- - - -
5	- - - -	† † - -	- - - -
6	- - - -	- - - -	- - - -
7	- - - -	† † † -	- - - -
8	- - - -	† † † -	- - - -
9	- - - -	† † † †	† † - -
10	- - - -	† † - -	† - - -
11	- - - -	† - - -	- - - -
12	- - - -	- - - -	- - - -
13	- - - -	- - - -	- - - -
15	- - - -	- - - -	- - - -

Tests of Native Sheep.

The blood of 20 sheep taken at the Jaffa Slaughter-House gave the following result (55% positive). These sera were only tested up to 1 : 200.

No.	*English*	*Local*	*Melitensis*
4	-	† †	-
6	-	† † † †	†
7	-	† † † -	†
8	-	† †	-
9	-	† †	-
11	-	† †	-
12	-	† † †	-
15	†	† † † †	† † †
17	-	† † † †	† † †
18	-	† † † †	† †
20	-	† † † †	† †

Tests of actually Aborting Cattle.

A number of tests of this nature have now been carried out with emulsions of the three types of Brucella, of which the following are examples :—

J/85 Holland Cow
E. — 1 : 200
L. — 1 : 1600
M. — 1 : 100

J/86 Holland Cow
E. — 1 : 50
L. — 1 : 100
M. — Negative

J/87 Native Cow
E. — 1 : 1600
L. — 1 : 400
M. — Negative

I/84 Native Cow
E. — 1 : 800
L. — 1 : 200
M. — 1 : 50

I/707 Native Cow
E. — 1 : 800
L. — 1 : 25
M. — Negative

P/416 Damascus Cow
E. — 1 : 200
L. — 1 : 1600
M. — 1 : 25

Interagglutination and Absorption Tests.

The emulsion used in all tests is one corresponding in opacity to a 1 : 3200 dilution of Barium Sulphate. A water clear reaction of a serum diluted 1 : 25 is read as positive. Highest titres are those which continue to be water clear.

A large number of absorption tests were carried out, but it is to be admitted that they showed some variability. The first marked variation was that naturally occurring cases in cattle showed a marked relationship between the English and Local strains, whereas in immune sera artificially prepared from other animals this relationship was reversed. For example, the sera of a rabbit and a goat immunised against Local strain gave the following titres :—

	Rabbit	*Goat*
English	1 : 100	1 : 400
Local	1 : 3200	1 : 2000
Melitensis	1 : 1600	1 : 1500

A selected negative native heifer in the earlier stages of immunisation reacted as follows :—

English	1 : 800
Local	1 : 800
Melitensis	1 : 200

With this animal titres fell rapidly and could only be maintained or slightly increased by repeated injections, finally reaching as much as 50 cc. of a heavy emulsion which was injected intravenously and intraperitoneally. The subsequent test was somewhat different, viz. :—

English	1 : 400
Local	1 : 1000
Melitensis	1 : 600

This may be accounted for by something in the nature of a pro-agglutinoid reaction arising from degeneration of agglutinins.

The goat's serum also tended to decrease rapidly in titre, and the following was arrived at :—

English	1 : 200
Local	1 : 1000
Melitensis	1 : 750

With these sera absorption tests were again carried out, using the standard technique described by Mackie and McCartney (Practical Bacteriology). The results are tabulated as follows :—

CALF ANTI-SERUM L.

		E.	*L.*	*M.*
Immune serum	Tested with	1 : 400	1 : 1000	1 : 600
,, absorbed by	E. ,, ,,	Negative	1 : 750	1 : 400
,, ,, ,,	L. ,, ,,	1 : 120	Neg.	Neg.
,, ,, ,,	M. ,, ,,	1 : 200	1 : 500	Neg.

GOAT ANTI-SERUM L.

		E.	*L.*	*M.*
Immune serum	Tested with	1 : 200	1 : 1000	1 : 750
,, absorbed by	E. ,, ,,	Neg.	1 : 750	1 : 600
,, ,, ,,	L. ,, ,,	1 : 100	Neg.	Neg.
,, ,, ,,	M. ,, ,,	1 : 150	1 : 300	Neg.

In general the results obtained by absorption tests carried out on the sera of various animals immunised against the Local strain showed a marked relationship between this strain and Br. melitensis.

I wish to express my thanks to the Laboratory Assistant, Mr. L. Smerkiss, for his care and attention in carrying out technical details.

Discussion.

It would appear from the above results that a strain of Brucella of low virulence as far as the lower animals are concerned* is prevalent in Palestine. It is capable of causing abortion of cattle, but has not yet given rise to severe outbreaks such as occur as a result of infection with typical European strains.

That little or no immunity exists in native cattle as a result of infection with the Local strain is shown by the following example :—

Several settlements are now being tested at short intervals under an agreement method of segregation control. At one of these purely native stock are being improved by crossing with pure bred Holland bulls. An extremely acute type of abortion has broken out, so acute in fact that abortions have frequently occurred a week after the animals have given a negative result, subsequently, of course, proving strongly positive.

The responsible organism has been isolated by guinea-pig inoculation with swab material and subsequent culture. In all respects it is identical with E.264 and on potato gives a typical chocolate-coloured growth. Some 10—15%, however, react quite independently to the Local strain.

In another settlement there are only pure bred Holland cattle. A few sporadic abortions have taken place in the last two or three years. About 15% of these cattle now react to the local strain, this percentage having increased during the last six months. There is no trace of these animals having been exposed to infection from other cattle, but for some time they were exercised on an adjacent hillside, which is a common pasturage for goats.

The strain under review appears to occupy a somewhat intermediate position between Br. melitensis and Br. abortus. Assuming that agglutination tests are a sufficiently accurate criterion of infection without confirmation by isolation of the organism, an attempt to carry out which has not been possible up to the present, sheep and goats would seem to be the common carriers. It is possible that some alteration in the characters of the organism might take place in its passage through cattle.

As to its practical importance to the dairy farmer, beyond occasional loss from abortion, further observation will have to be made as to sterility, effect on calves, or the possibility of increase in virulence.

References.

[1] Good, Dimoch, and Harms. Journal of American Veterinary Medical Association, Volume 73, page 223.

[2] Traum and Henry. Extract in Tropical Veterinary Bulletin, Volume 17, No. 4.

[3] Stuart. Extract in Tropical Veterinary Bulletin, Volume 14, No. 1.

[4] McFadyean. Journal Comparative Pathology and Therapeutics, Volume XLI, Part 4, page 362.

* The author passed through a fairly severe attack of Undulant Fever some seven years ago. His serum now gives a positive result at a dilution of 1:25 to the Br. melitensis and up to 1:200 with the Local strain described.

"KEEL DISEASE" IN DUCKLINGS IN BRITAIN.

By S. H. Gaiger and G. O. Davies

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The writers have studied an outbreak of " Keel Disease " in ducklings in this country. Their attention was drawn to this outbreak by Mr. G. C. Marginson, M.R.C.V.S., to whom they are indebted for the material and for the information regarding the epizootiology. The name " Keel Disease " was given to the outbreak because of its clinical similarity to a condition first so described by Rettger and Scoville[1] in America in 1920. The circumstances with regard to the present outbreak were as follows.

The outbreak occurred on a farm where 25,000 ducklings were being reared. These ducklings were bought when two or three days old from a farm a few miles away where they were being hatched and on which the adult stock was apparently quite healthy. The breeder of the ducklings regularly sold them also to other farms where they remained perfectly healthy, yet the eggs were from the same stock as the eggs whose ducklings went to the farm on which the disease occurred.

On the affected farm (Farm A) the ducklings were penned off in the open, about 200 for each pen. They were kept in cabins containing brooders during the night time, and were let out daily into the pens. The ducklings were fed every few hours with thirds, bran, low-grade flour, barley meal and fish meal ; the drinking water was obtained from a pit on the farm. The ducklings on the farm ranged between the ages of a day or two and nine weeks.

The disease affected ducklings between one and three weeks old, many dying daily. Four thousand ducklings had been lost from the disease during 1929. The symptoms commenced with loss of appetite, a slight discharge from the eyes and nose, constipation, and in some cases slight diarrhœa. There quickly followed an inability to co-ordinate the movement of the legs, the ducklings easily falling over and being unable to rise again, lying on their backs on the ground, kicking their legs in an attempt to turn over and get up again. Some of the affected showed signs of the brain being involved, in that they walked with their heads on one side, turned round in circles, and banged into objects as if they were blind. Death usually occurred within 24 hours of the onset of symptoms in the younger ducklings, but others lived for some days. On each visit to the farm a large percentage of the remaining ducklings were found showing symptoms of disease. The older ducks (three to nine weeks) on the diseased farm appeared healthy apart from a slight discharge from the eyes which matted the feathers. As the disease had been going on amongst the ducklings during the whole summer, it appeared that these older ducks must have come from pens in which many of their fellows had died at an early age.

With each batch of healthy ducklings brought from the breeder's farm (Farm B) to the affected farm, deaths began a few days after arrival, and increased in number until about the seventeenth day. About 80 per cent. of the diseased ducklings died, and the remainder, after recovery, were in very poor condition for several weeks. During the two or three days that the ducklings remained on Farm B prior to being brought to Farm A, they were kept under similar conditions as regards housing at night and being let out in the daytime. Their food consisted of bran, flaked corn, and thirds. The owner of the diseased ducklings was of opinion that the food was the predisposing cause of the trouble, as he found that his men were mixing it and allowing it to stand in bins for some time before feeding, thus allowing fermentation to take place. The farm on which the outbreak occurred had been used continuously for the rearing of ducklings for several years without anything similar to the outbreak of this year having occurred.

During the course of the outbreak the food and method of feeding were altered, and tap water was used in place of the pit water, but deaths continued to occur in the same large numbers. As the disease had been present during the year and infection distributed over a large part of the farm, it appeared impracticable to adopt disinfection measures. Some of the ducklings were removed to fresh ground, and another lot was removed to land surrounding the pit from which the drinking water had been obtained. Nevertheless, deaths continued to occur on both these sites. On this account no more young ducklings were brought on to Farm A for a time, the lots which would have been brought being kept on Farm B to ascertain whether disease began in them when retained beyond the customary periods on this farm. The ducklings, however, remained perfectly healthy on Farm B. It therefore appeared certain that the ducklings acquired infection on Farm A, and did not bring it with them. When these ducklings, kept upon Farm B, were a fortnight old, about 1,000 of them were brought to Farm A. Of these, 200 were placed in diseased pens which had not been disinfected, while the remainder were placed in clean pens not previously used for ducks and attended by men who had never been in contact with the diseased ducklings. By the time these 1,000 ducklings had been on Farm A for two weeks, making them four weeks old, less than 5 per cent. of deaths had occurred, which is considered the normal death-rate in the rearing of young ducklings. Amongst this 5 per cent. none showed symptoms of " Keel Disease," and the death-rate was the same amongst those placed in the infected pens as amongst those in clean pens.

From these field observations it was apparent that the infection was picked up on Farm A during the first fortnight of life. The observations did not lend any support to the supposition that infection was present in the eggs from possible adult carriers. At this stage about 3,000 ducklings were placed in the previously infected pens at a week old, and another 3,000 at two days old. " Keel disease " failed to appear amongst the 6,000 ducklings. From this it was supposed either that infection must have quickly died down upon

Farm A in the absence of infected ducklings to keep up the virulence, or that some predisposing factor such as improper feeding, which had previously operated, had now been removed. A number of live ducklings were brought to the laboratory of the Department of Veterinary Pathology for examination, and these were killed and carefully examined post-mortem. There seemed to be a complete absence of lesions which would account for death. Some showed no diarrhœa at all, others showed slight signs of it, but there was no gastro-enteritis present. A careful bacteriological study was made of several of these ducklings, and the results will presently be detailed.

On Farm A it was reported that a considerable number of pigeons had been lost after showing symptoms of diarrhœa. The bacteriological findings in a killed pigeon will also be described.

" Keel Disease " in America.

In the 1918 outbreak described by Rettger and Scoville[1] in America, there were several points of resemblance to the outbreak described here. In the American outbreak the mortality approximated to 90 per cent. The symptoms described appear to be identical with the exception that in Rettger and Scoville's outbreak it is stated that some of the ducklings died soon after leaving the shell. This, of course, might have happened in the English outbreak had ducklings been hatched out on the affected farm. The name " Keel Disease " was given to the condition in America because the affected ducks showed as their principal symptom a " keeling " over, lying feet uppermost. This description exactly fits the symptoms as shown in the English outbreak. In the American outbreak post-mortem lesions were conspicuous by their absence. The American authors regarded the disease as being of an intestinal nature, although little or no diarrhœa was observed. Incidentally, the American authors remark on their surprise at the magnitude of the duck farming industry in America. Similar surprise was felt by the writers at the enormous number of ducklings raised on certain farms in this country.

Rettger and Scoville, using the ordinary laboratory media, had no difficulty in isolating from the liver, heart, and lungs of affected ducklings an organism belonging to the coli-typhoid group. They made careful study of the organism isolated, considering that it was a new species belonging to the Salmonella group, and gave it the name of *Bacterium anatum*. They state that Cornil and Toupet[2], in 1888, named an organism which they isolated from diseased ducks as *Bacterium anatis*, but a perusal of the article by Cornil and Toupet reveals no mention of this name, though an organism is described which Cornil and Toupet considered indistinguishable from *Bacillus avisepticus*. Rettger and Scoville further state that the organism from the ducklings affected with " Keel disease " bore a close resemblance to *Salmonella pullorum*, though the reason for this statement is far from apparent in their bacteriological findings. They examined eight breeding ducks, and in the ovary of one duck found three abnormal cysts, which had once been ova, containing dry yolk-like material in an amber-coloured clear fluid, the largest of these cysts being the size of a pea. From these cysts of this one duck the *Bacterium anatum* was obtained. In seven other ducks the ovaries were normal, but in one of these seven there was a cheesy abscess in the omentum which yielded pure cultures. These findings, in our view, would hardly justify the authors in making a general statement in the summary that " the organism was obtained in pure culture also from ovarian cysts and from an abdominal cyst of breeding ducks," nor would their statement appear to be justified that " attempts to fully demonstrate transmission of the

disease from the ovary through the egg to the offspring were not as successful, therefore, as they would have been had they begun early in the spring of the year."

Rettger and Scoville state that different strains of their organism revealed "slight and insignificant differences among themselves," though all belonged to the paratyphoid group, the differences being seen in the fermentation of certain sugars. Agglutination tests showed a close relationship between *B. anatum* and *B. paratyphosus B.*

B. anatum was found to be fatal on the third day to chicks about three weeks old after subcutaneous injection, and the organism could be recovered from the liver and heart blood. A local abscess developed after subcutaneous injection of a rabbit and a guinea-pig, but *B. anatum* was not grown from the pus. Fourteen ducklings of two days old were given agar surface culture emulsion subcutaneously. Some of these developed "keel" symptoms, but the last survivor showed a local abscess like the rabbit and guinea-pig. *B. anatum* was recovered from all the dead ducklings.

Edwards and Rettger[3], in 1927, further examined Rettger and Scoville's strains, and found them divisible into two types, one identical with *S. ærtrycke*, and the other a new species for which the name *S. anatum* was retained.

They further record that three large outbreaks of "keel disease" were met with in 1920 in America, the first recorded outbreaks being in 1918.

They examined ten strains from the various outbreaks by the agglutination, agglutinin-absorption, and complement fixation tests, and found that seven strains were *S. ærtrycke*, and three were *S. anatum*. The latter were "not fatal to rabbits upon injection."

Edwards[4], in 1929, found *S. anatum* associated with *S. ærtrycke* in a disease of chicks in which there was a mortality of 25 per cent. Edwards states that the symptoms closely resembled bacillary white diarrhœa, though there was no diarrhœa or any characteristic symptom. Edwards failed to demonstrate any adult carriers in the breeding stock, and examination of known survivors by post-mortem and the agglutination test also failed to reveal any carriers. Edwards therefore holds the view that this disease of chicks is contracted after hatching.

Doyle[5], in 1927, described a *S. ærtrycke* infection in chicks in Surrey, and states that a heavy mortality (69 per cent.) also occurred amongst young ducks, but none of the ducks were obtained for examination. The farmer was of opinion that the ducks died of the same disease as the chicks.

From this review of literature it will be seen (1) that several outbreaks of "keel" disease in ducklings have been met with in America, though the condition has not hitherto been described in Britain; (2) that a new organism—*Salmonella anatum*—belonging to the Salmonella group, has been found in association with *Salmonella ærtrycke* in connection with these outbreaks; (3) that the same associated organisms have been found in America causing deaths in chicks; (4) that the evidence seems to indicate that infection takes place after hatching; and (5) that *S. ærtrycke* has been recorded in England as the cause of deaths in chicks in an outbreak where heavy mortality in ducks was occurring, though in the latter species the causal organism was not investigated.

The following is a record of the laboratory studies in the outbreak herein recorded in ducklings in Lancashire.

September 13th, 1929. 5 p.m. Several ducklings, alive and dead, were received. Ducklings 1, 2, and 3 were selected as typical cases for study.

They were destroyed by breaking the cervical vertebræ and opened with the strictest aseptic precautions. Cultures were made on agar, serum agar, nasgar, solidified serum, and bouillon, from the liver pulp and heart blood of each. In none were lesions found on post-mortem which might account for the symptoms of disease.

September 14th, 1929. 10 a.m. The cultures had been at this time 16 hours in the incubator. Those made from Ducklings 1 and 3 were all negative.

Duckling 2.—Bouillon cultures from heart blood and liver were turbid. Nasgar, serum agar, and solid serum media sown from the liver showed pin-head, dew-drop-like translucent and glistening colonies. The same media from the heart blood were negative.

Microscopic examination made it apparent that more than one organism was present. In the plain bouillon tubes there was an apparently pure growth of a Gram-negative motile bacillus, of varying length, with rounded ends, staining faintly and resembling organisms of the coli-typhoid group.

Subsequent further incubation of the nasgar and serum tubes revealed a fine film between some of the coli-like colonies, and an examination of this showed a very much longer and thinner, curved, faintly staining, Gram-negative, non-motile bacillus.

These two organisms were later separated in subculture in a state of purity.

On September 16th, 1929, the cultures from Duckling 3 showed growths on all media from the heart blood and liver parenchyma. Nasgar, serum media, and one plain agar tube from the heart blood yielded the fine colonies referred to in Duckling 2 in apparently pure culture and free from coli-type colonies visible to the naked eye. All the liver parenchyma tubes showed the fine colonies, but in two tubes isolated colonies of the coli-type were present visible to the naked eye.

Microscopic examination of the growths revealed that the two organisms from Duckling 3 possessed the same general characters as those cited for Duckling 2. In several tubes the two organisms were found on microscopic examination to be growing side by side on the same medium, although the coli-type colonies had not assumed naked-eye dimensions.

Continued incubation of the original tubes and repeated subculturing from them showed that all the heart blood cultures were apparently pure cultures of the fine-colony organism, whereas several of the liver tubes subsequently developed the coli-type colonies visible to the naked eye.

Duckling 1 yielded no growths of bacteria on any media, and the tubes were discarded.

On September 19th, 1929, three more sick ducklings (Ducklings 4, 5 and 6) and a sick pigeon (Pigeon 1) were received from Farm A, and were killed and dealt with in the same manner as previously.

Duckling 4, by September 25th, showed no colonies of any organism on any medium, and the tubes were discarded.

Duckling 5 gave growths of the coli-type organism from the heart blood on plain agar, and from the liver the same organism was obtained on nasgar and solid serum without any colonies of the fine-colony bacillus.

Duckling 6 yielded from the liver parenchyma colonies of the coli-type bacillus, and in addition " brown colonies " of a non-motile, Gram-negative bacillus tending to form short curved filaments, the curves being of a half circle or a nearly complete circle, and showing a granular streptococcus-like appearance with Loeffler's alkaline methylene blue. The " brown-colony " organisms only grew in the original solid serum tubes, and the colonies were

rather mucoid in consistence, tending to liquefy the serum. Subcultures on blood agar were faint in 24 hours, but heavier in 48 hours.

From the heart blood the coli-type bacillus was not obtained, but the " brown colony " bacillus was obtained as a very faint growth after six days' incubation, both on serum agar and plain agar, growing better in subculture on blood agar and solid serum. It was at first thought that the " brown colony " bacillus might be a contaminant from the serum, but its appearance also on the original plain agar tubes showed that the organism must have been present in the sick duckling.

Pigeon 1 yielded a coli-type organism on all media from both heart blood and liver.

From the above cultural study the following organisms were kept in subculture for further study :

Duckling 2.—Coli type.
„ 3.—Serophile type.
„ 3.—Coli type.
„ 5.—Coli type.
„ 6.—Coli type.
„ 6.—Brown serophile type.
Pigeon 1.—Coli type.

At this early stage in the investigation the " fine colonies " serophile bacillus was regarded as possibly the causal organism, if the disease were really a bacterial one, and the pathogenicity was tested as shown in the following table. It was, unfortunately, impossible to obtain young ducklings at that time for the experiments.

TABLE I.

Animal.	*Inoculum.*	*Dose.*	*Method.*	*Result.*
Rabbit ...	" Fine colonies " (D. 3) serum-agar 48 hours culture, One whole agar culture in 2·75 c.c. normal saline solution.	0·5 c.c.	Subcut.	No result
Rabbit ...		0·25 „	Intrav.	No result
Guinea-pig (G. 7).		0·5 „	Subcut.	Dead in 5 days.
Guinea-pig		0·5 „	Intraper.	No result
Pigeon ...		0·5 „	Intramusc.	No result
Pigeon ...		0·5 „	Intramusc.	No result

The rabbits and pigeons were the youngest obtainable, and were probably not under six months old.

The post-mortem examination of guinea-pig G. 7 revealed extensive lesions of a pseudo-tuberculosis in the liver, sublumbar glands, and precrural glands. The spleen was enlarged and fibrous. It was concluded that this condition was an old-standing infection which had no connection with the inoculation. Cultures were made from the heart blood and liver of this guinea-pig, and yielded apparently pure and very massive cultures of a coli-type organism grown from Duckling 3 and other ducklings. It seemed that the explanation of this result was that the apparently pure " serophile " culture was contaminated to a small degree with the soli-type bacillus which, in an already-diseased guinea-pig, was able to set up a fatal infection.

TABLE II.

	S.T. D. 2.	*Serophile D.* 3.	*S.T. D.* 5.	*S.T. D.* 6.	"*Brown Colonies*" *D.* 6.	*S.T. Pigeon* (*P.* 1).
Glucose	A. and G.	—	A. and G.	A. and G.	—	A. and G.
Lactose	N.C.	—	N.C.	N.C.	—	N.C.
Maltose	A. and G.	—	A. and G.	A. and G.	—	A. and G.
Saccharose	A. and G.	—	Minute bubble. No colour change.	N.C.	—	N.C.
Mannite	A. and G.	—	A. and G.	A. and G.	—	A. and G.
Sorbite	A. and G.	—	A. and G.	A. and G.	—	A. and G.
Raffinose	N.C.	—	N.C.	N.C.	—	Not inoculated.
Lævulose	A. and G.	—	A. and G.	A. and G.	—	Do.
Salicin	N.C.	—	N.C.	N.C.	—	N.C.
Galactose	A. and G.	—	A. and G.	A. and G.	—	A. and G.
48th hour	All same. Good growth in negative tubes.	Same. No growth in medium.	Same. Negative tubes good growth.	Same. Negative tubes good growth.	No sign of growth in the medium.	Same. Negative tubes good growth.
96th hour	Same, but Maltose and Sorbite green from yellow.	Same.	Same, but Sorbite changing green from yellow.	Same, but Sorbite changing green from yellow.	Growth in Sorbite of stringy appearance. Acid, but no gas.	Same.

A. = Acid. G. = Gas. N.C. = No change.

The failure of this serophile organism to show any pathogenicity led us to pay more attention to what had hitherto been regarded as a coli-type organism, and which had been isolated from Ducklings 2, 3, 5, and 6, the pigeon, and Guinea-pig G. 7. This organism was grown on some of the sugars, and found to be a non-lactose-fermenter, thus bringing it into the Salmonella group. It is hereafter referred to as bacillus S.T. The sugar fermentation reactions of the three organisms obtained from the ducklings are shown in Table 2. (see page 131.) The sugar medium indicator used was bromthymol blue.

The pathogenicity of the " brown colonies " serophile organism was then tested :

TABLE III.

Animal.	*Inoculum.*	*Dose.*	*Method.*	*Result.*
Pigeon 36	48 hour cultures on blood-agar.	Each received ½ culture suspended in Saline.	Subcut.	No result
Pigeon 37	Do.		Intramusc.	No result
Rabbit 38	Do.		Subcut.	No result
Guinea-pig 39.	Do.		Subcut.	No result

Preliminary pathogenicity tests were made with Bacillus S.T.

TABLE III.

Animal.	*Inoculum.*	*Dose.*	*Method.*	*Result.*
Guinea-pig G. 11.	Heavy emulsion from a 48 hour culture, from Duckling 5.	0·5 c.c.	Subcut.	Death with local œdema and peritonitis.
Pigeon G.12	Do.	0·5 ,,	Intramusc.	No result.
Pigeon G.13	Do.	1·0 ,,	Intramusc.	No result.

Cultures from the heart blood and liver of Guinea-pig G. 11 yielded the bacillus S.T. in pure culture. The sugar fermentation reactions of the organism resembled those of Bacillus S.T. (D. 5) given on Table 2, with the exception of acid and gas production in saccharose after 24 hours' incubation. The variable fermentation results of this sugar will be referred to later.

The recovery from two inoculated guinea-pigs of coliform organisms which were apparently identical with those originally isolated from the ducklings, together with the absence of pathogenicity displayed by the serophile organism previously mentioned, led us to regard this coliform organism or Bacillus S.T., as we now refer to it, as the possible cause of the disease in the ducklings.

Reference was consequently made to Bergey's " Manual of Determinative Bacteriology," and under *Salmonella anatum* (Rettger & Scoville) there was described an organism which appeared to resemble very closely the bacillus which we had isolated. With this in mind we decided to continue our studies of the general characteristics of the original strains of Bacillus S.T. Further pathogenicity experiments were conducted, and comparisons were made of the sugar fermentation reactions of our original strains, the organisms recovered following animal inoculation, and Rettger's strains which we were able to procure from the National Collection of Type Cultures.

Attempts were also made to produce immune sera from rabbits for purposes of agglutination, and by that means determine the relationship of Bacillus S.T. to members of the Salmonella Group. The details of these experiments will be arranged, where possible, in tabular form.

General Characters of Bacillus S.T.

The organism is a short, Gram-negative, round-ended bacillus, which varies in length. Cocco-bacillary forms are often present, and long bacillary forms are not infrequent. It is an ærobe and facultative anærobe, and, like members of the colon-typhoid group, grows very readily on ordinary laboratory media, the optimum temperature being about 37° C. On agar and nasgar in subcultures one gets a heavy growth along the needle track in 16-24 hours. Broth becomes turbid in a few hours, often with a good growth on the side of the tube at the level of the medium, but no surface pellicle is formed. A very abundant growth takes place in gelatin at 37° C., but the gelatin solidifies when exposed at room temperature. In a gelatin stab there develops a uniform growth along the needle track, and occasionally the medium is broken up in one or two places. On blood media slight hæmolysis takes place. Indol is not formed, and numerous sugars are fermented with the production of acid and gas.

Pathogenicity.

All emulsions (except where mentioned) used in the following pathogenicity experiments were standardised to the density of Brown's opacity tube No. 10.

Two of Rettger's non-ærtrycke strains, *B. Anatum*, C. 1 and C. 5, were received from the National Collection of Type Cultures, and it was decided to compare the results of their inoculation into guinea-pigs with the results tabulated in Table IV. (see page 134.)

It will be seen from Table IV that attempts to infect 10 days old chicks and 3 weeks old ducklings did not meet with success when the emulsion was administered either orally or subcutaneously. Pigeons were also refractory when tried by subcutaneous and intramuscular routes. Mice were more susceptible, fatal results following subcutaneous inoculation in four out of six cases. On post-mortem examination there was a marked absence of macroscopic lesions. Congestion of the internal organs with a slight increase of fluids in the serous cavities was occasionally present. There was never any difficulty experienced in recovering the inoculated organism from the heart blood and liver. In guinea-pigs and rabbits the results were very similar. Large doses subcutaneously were invariably followed by fatal results, whereas small doses gave rise to abscesses at the site of inoculation. An organism identical with that inoculated was isolated from the internal organs of all the animals that died, and in the other cases from the abscess at the inoculation site when cultures were attempted.

In the comparative experiments (Table V) performed with Rettger's *B. anatum*, strains C. 1 and C. 5, the results displayed a

TABLE IV.

Animal.	*Inoculum.*	*Dose.*	*Method.*	*Result.*
Mouse No. 40	Bacillus S.T. (D. 5) emulsion	0·1 c.c.	Subcut. ...	Died 2nd day. Organism recovered.
Mouse No. 41	Do.	Do.	Do. ...	Died 4th day. Organism recovered.
Mouse No. 42	Do.	Do.	Do. ...	Died 6th day. Organism recovered.
Mouse No. 43	Do.	Do.	Do. ...	Died 10th day. Organism recovered.
Mouse No. 44	Do.	Do.	Do. ...	Negative.
Mouse No. 45	Do.	Do.	Do. ...	Do.
Pigeon No. 46	Do.	0·25 c.c.	Intramusc.	Do.
Pigeon No. 47	Do.	Do.	Do. ...	Do.
Pigeon No. 48	Do.	Do.	Subcut. ...	Do.
Pigeon No. 49	Do.	Do.	Do. ...	Do.
Rabbit No. 50	Do.	Do.	Do. ...	Local abscess from which organism was recovered on 11th day.
Rabbit No. 51	Do.	1·0 c.c.	Do. ...	Killed 11th day at point of death, local abscess from which organism was recovered.
Guinea-pig No. 52 ...	Do.	0·25 c.c.	Do. ...	Local abscess. Animal recovered.
Guinea-pig No. 53 ...	Do.	1·0 c.c.	Do. ...	Died 10th day. Organism recovered from heart blood, liver, thoracic cavity, and suprarenal gland.
Duckling No. 54	S.T. (D. 5) emulsion... ...	2 c.c.	Orally ...	Negative.
Duckling No. 55	Do.	0·5 c.c.	Subcut. ...	Negative apart from initial dullness.
Duckling No. 54	Very heavy S.T. (D. 5) emulsion.	0·5 c.c. and 0·5 c.c.	Do. ...	
Do. (2nd dose)	Do.	Do.	Orally ...	Negative.
Duckling No. 55... ...	Do.	Do.	Subcut. ...	
Do. (2nd dose)	Do.	Do.	Orally ...	Do.
Chick No. 68 (10 days old)	S.T. (D. 5) emulsion ...	0·25 c.c. and 0·25 c.c.	Subcut. ...	
			Orally ...	Do.
Chick No. 69 (10 days old)	Do.	Do.	Subcut. ...	
			Orally ...	Do.
Guinea-pig No. 82 ...	Bacillus S.T. (P. 1) emulsion	0·25 c.c.	Subcut. ...	Local abscess. Organism recovered.
Guinea-pig No. 83 ...	Do.	1·0 c.c.	Do. ...	Died 2nd-3rd day. Organism recovered from heart blood, gall bladder, and spleen.
Guinea-pig No. 92 ...	Bacillus S.T. (D. 2) emulsion	Do.	Do. ...	Died 11th day. Organism recovered from local abscess, heart blood, and liver.
Guinea-pig No. 93 ...	Do.	0·25 c.c.	Do. ...	Local abscess. Organism recovered.
Guinea-pig No. 94 ...	Bacillus S.T. (D. 6) emulsion	1·0 c.c.	Do. ...	Died 6th day. Organism recovered from local abscess, heart blood and liver.
Guinea-pig No. 95 ...	Do.	0·25 c.c.	Do. ...	Local abscess. Organism recovered.

marked similarity. The inoculations were followed by similar lesions, but they were not so extensive or fatal as with our S.T. strains, which we attributed to their attenuation as a result of prolonged subcultivation.

Biochemical Reactions. Fermentation of Sugars.

We have already recorded in Table II the sugar fermentation reactions of the three original strains of Bacillus S.T. isolated from ducklings (D. 2, D. 5, and D. 6), and also the strain from the pigeon (P. 1). In Table VI we summarise further fermentation reactions, which were conducted with Bacillus S.T. (D. 5), and also include for comparative purposes the results which we obtained with four strains of *B. anatum* (Rettger). The particulars of these strains are :

(1) Salmonella anatum (Rettger C. 1)—non-aertrycke type.
(2) Salmonella anatum (Rettger C. 5)—non-aertrycke type.
(3) Salmonella anatum (Rettger 850)—aertrycke type.
(4) Salmonella anatum (Rettger 851)—aertrycke type.

The sugars used throughout all the tests were prepared according to Christiansen's method[6], using sugar in ½ per cent. quantities and bromthymol blue as indicator. The test was carried out at 37° C., and acid production was indicated by the medium assuming a yellow colour. The reactions were read daily, but those recorded were taken on the fourth day.

In considering the results (page 136) the most marked feature is the agreement which is displayed by all the strains. It is evident that the Bacillus S.T. and the " aertrycke " and " non-aertrycke " types of *B. anatum* are indistinguishable by sugar reactions. Acid and gas are produced by all strains in glucose, lævulose, galactose, maltose, and mannite, whereas no fermentation takes place in lactose, inulin, adonite, and salicin. In sorbite the preliminary acid and gas production is marked, but between the twenty-fourth and seventy-second hour one usually sees taking place a gradual change in colour from yellow to the initial blue green, the gas production remaining unaltered. Consequently, readings taken daily during the first four days vary according to the amount of acid present, and sometimes it will even be noticed that on the fourth day the change is not complete. Variations in the results recorded for sorbite can thus be explained.

Saccharose, arabinose, dextrin, xylose, and inosite gave irregular results. These differences could not be attributed to the strains, as the variations occurred in subsequent tests with the same organisms. Edwards and Rettger[3] report variable results in the fermentative tests of their several strains of *B. anatum*. This they attribute in some cases to the lack of a " favourable nitrogenous substrate," which, as in the case of xylose, was responsible for gas production in one medium and its absence in another. It is conceivable that the irregularities in our strains and the slight differences between their results and ours may be due to the same factor.

It will be observed that the strains used in the pathogenicity experiments, were those originally isolated, viz. : Bacillus S.T.—D. 2, D. 5, D. 6, and P. 1. A comparison of the sugar reactions of

TABLE V.

Animal.	*Inoculum.*	*Dose.*	*Method.*	*Result.*
Guinea-pig No. 88 ...	Emulsion B. Anatum (Rettger C. 1).	1·0 c.c.	Subcut. ...	Animal killed owing to large local abscess, from which organism was recovered.
Guinea-pig No. 89 ...	Do.	0·25 c.c.	Do. ...	Small abscess at site of inoculation.
Guinea-pig No. 90 ...	Emulsion B. Anatum (Rettger C. 5).	1·0 c.c.	Do. ...	Local abscess. No cultures attempted.
Guinea-pig No. 91 ...	Do.	0·25 c.c.	Do. ...	Local abscess. Organism recovered.

TABLE VI

	Salmonella S.T. (D. 5).		*B. Anatum (Rettger) C. 1.*		*B. Anatum (Rettger) C. 5.*	*B. Anatum (Rettger) Aertrycke 850.*		*B. Anatum (Rettger) Aertrycke 851.*
	1st Test.	*2nd Test.*	*1st Test.*	*2nd Test.*		*1st Test.*	*2nd Test.*	
Glucose	A. and G.	A. and G.	A. and G.	A. and G.	A. and G.	A. and G.	A. and G.	A. and G.
Lævulose...	A. and G.	A. and G.	A. and G.	A. and G.	A. and G.	A. and G.	A. and G.	A. and G.
Arabinose	A. and G.	*G.	A. and G.	—	—	A. and G.	*G.	*G.
Galactose	—	A. and G.	—	—	—	—	A. and G.	A. and G.
Xylose	A. and G.	S.A. and G.	A. and G.	—	—	A. and G.	*G.	A. and G.
Lactose	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.
Maltose	A. and G.	A. and G.	A. and G.	A. and G.	A. and G.	A. and G.	A. and G.	A. and G.
Saccharose	*G.	N.C.	*G.	*G.	*G.	*G.	N.C.	N.C.
Inulin	N.C.	N.C.	N.C.	—	—	N.C.	N.C.	N.C.
Dextrin	N.C.	*S.G.	N.C.	—	—	N.C.	*S.G.	*S.G.
Adonite	N.C.	N.C.	N.C.	—	—	N.C.	N.C.	N.C.
Mannite	A. and G.	A. and G.	A. and G.	A. and G.	A. and G.	A. and G.	A. and G.	A. and G.
Sorbite	*G.	*G.	*G.	*G.	*G.	*G.	*G.	*G.
Salicin	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.	N.C.
Inosite	N.C.	N.C.	N.C.	—	—	S.A.	N.C.	N.C.

A. = Acid production. G. = Gas production. — = Not tested.
N.C. = No change. S.G. = Slight gas. S.A. = Slight acid.
* = Yellow coloration at first which has later changed to original blue green.

the organisms recovered from inoculated animals with the standards which we had established was used in establishing their identity. Table VII comprises the results of the individual tests. Only those sugars which were known to give reliable results were used, and unfortunately in some instances complete sets were not available when required.

Agglutination Tests.

Rabbits were chosen for the preparation of antisera and standard suspensions were used as in the pathogenicity experiments. Twenty-four-hour agar slant cultures were washed off with carbolised saline in the case of suspensions to be killed later by heat, and normal saline in the case of living suspensions. These suspensions were then standardised to the density of Brown's standard opacity tube No. 10.

Our earlier attempts were not very successful and, furthermore, it was very difficult to account either for the variable susceptibilities of the rabbits or for the apparent fluctuations in the toxicity of the antigen. The details are as follows :

(1) *Rabbit* 56.—Inoculated subcutaneously 0·5 c.c. living Bacillus S.T. emulsion. Six days later : Inoculated intravenously 0·25 c.c. living Bacillus S.T. emulsion.

The rabbit was found dead next day, and the bacillus was recovered in culture from the internal organs.

(2) *Rabbit* 77.—Inoculated subcutaneously 0·25 c.c. killed Bacillus S.T. emulsion. Inoculated six days later intravenously 0·25 c.c. killed Bacillus S.T. emulsion. Inoculated four days later intravenously 0·2 c.c. living Bacillus S.T. emulsion.

Rabbit died on the third day following the last inoculation, and the bacillus was recovered in culture.

(3) *Rabbit* 50.—This rabbit had recovered from a subcutaneous inoculation of 0·25 c.c. of living Bacillus S.T. emulsion, and was inoculated one month later intravenously with 0·1 c.c. killed Bacillus S.T. emulsion. It was also inoculated four days later intravenously with 0·2 c.c. killed Bacillus S.T. emulsion.

The rabbit died three days later, and the bacillus was recovered in culture.

(4) *Rabbit* 87.—Inoculated subcutaneously 0·2 c.c. killed culture Bacillus S.T. emulsion, and six days later intravenously 0·1 c.c. killed culture of the same organism. The rabbit died the following day. Cultures were all negative.

Following these unsuccessful attempts, we decided to confine ourselves to the use of dead emulsions subcutaneously. Two rabbits, Nos. 104 and 105, were respectively immunised by gradually increasing doses of Bacillus S.T. emulsion and *Salmonella anatum* C. 1 (Rettger) emulsion. The rabbits were inoculated at from four to six-day intervals with doses of killed emulsion ranging from 0·5 c.c. to 1·25 c.c. They each received seven subcutaneous inoculations before blood was finally collected for the agglutination test. Blood was previously collected from each of the rabbits following the fourth subcutaneous inoculation. The results of the tests conducted with these sera are shown in Tables VIII and IX. Agglutination tests were also carried out with Bacillus S.T. emulsion and *Salmonella ærtrycke*, *Bacillus paratyphosus A*, and *Bacillus paratyphosus B* sera.

TABLE VII.

Animal.	*Strain.*	*Glucose.*	*Lævulose.*	*Lactose.*	*Maltose.*	*Saccharose.*	*Mannite.*	*Sorbite.*	*Salicin.*
Guinea-pig No. 11	S.T. D. 5	A. and G.	A. and G.	N.C.	A. and G.	*S.G.	A. and G.	*G.	N.C.
Guinea-pig No. 7	S.T. D. 5	A. and G.	A. and G.	N.C.	A. and G.	*S.G.	A. and G.	*G.	N.C.
Mouse No. 1	S.T. D. 5	A. and G.	—	N.C.	A. and G.	—	—	*G.	N.C.
Mouse No. 2	S.T. D. 5	A. and G.	—	N.C.	A. and G.	—	—	*G.	N.C.
Mouse No. 4	S.T. D. 5	A. and G.	—	N.C.	A. and G.	—	—	*G.	N.C.
Guinea-pig No. 53	S.T. D. 5	A. and G.	—	N.C.	A. and G.	—	—	*G.	N.C.
Rabbit No. 50	S.T. D. 5	A. and G.	—	N.C.	A. and G.	—	—	*G.	N.C.
Rabbit No. 51	S.T. D. 5	A. and G.	—	N.C.	A. and G.	—	—	*G.	N.C.
Rabbit No. 72	S.T. D. 5	A. and G.	—	N.C.	A. and G.	—	—	*G.	N.C.
Guinea-pig No. 83	S.T. P. 1	A. and G.	A. and G.	N.C.	A. and G.		A. and G.	*G.	N.C.
Rabbit No. 50 (2nd inoculation)	S.T. D. 5	A. and G.	A. and G.	N.C.	A. and G.	*G.	A. and G.	*G.	N.C.
Guinea-pig No. 82	S.T. P. 1	A. and G.	A. and G.	N.C.	A. and G.	*G.	A. and G.	S.A. and G.	N.C.
Rabbit No. 77	S.T. D. 5	A. and G.	A. and G.	N.C.	A. and G.	*G.	A. and G.	A. and G.	N.C.
Guinea-pig No. 94	S.T. D. 6	A. and G.	A. and G.	N.C.	A. and G.	*G.	A. and G.	*G.	N.C.
Guinea-pig No. 92	S.T. D. 2	A. and G.	A. and G.	N.C.	A. and G.	*G.	A. and G.	*G.	N.C.
Guinea-pig No. 95	S.T. D. 6	A. and G.	A. and G.	N.C.	A. and G.	*G.	A. and G.	*G.	N.C.
Guinea-pig No. 88	Rettger C. 1.	A. and G.	A. and G.	N.C.	A. and G.	N.C.	A. and G.	*G.	N.C.
Guinea-pig No. 91	Rettger C. 5.	A. and G.	A. and G.	N.C.	A. and G.	N.C.	A. and G.	*G.	N.C.

Symbols used here are the same as noted for Table VI.

The antigens used in the agglutination tests were prepared by suspending in carbolised saline twenty-four-hour cultures of the respective organisms on agar media. The suspensions were then filtered and standardised to a density of Brown's opacity tube No. 2. Agglutination tubes were incubated at 37° C. for 24 hours, and the readings were taken after they had been allowed to stand at room temperature for two hours.

Serum.	*Antigen.*	*Results.*
B. ærtrycke	Bacillus S.T.	Negative in 1 : 20 dilutions
B. paratyphosus A.	Do.	Do.
B. paratyphosus B.	Do.	Do.

TABLE VIII.

Serum.	*Antigen.*	*Serum Dilutions.*						
		25.	50.	100.	200.	400.	800.	1600.
Bac. S.T. (D.5) Serum.	Bac. S.T. (D. 5).	+	+	+	+	+	+	—
Do. (after four inoculations of killed culture.	Salmonella Anatum C. 1 (Rettger).	++	++	++	++	++	+	+
Salmonella anatum (Rettger) C. 1.	Bac. S.T. (C. 5).	+	+	+	+	+	+	—
Do. after four inoculations of killed culture).	Salmonella anatum. C. 1 (Rettger).	++	++	++	++	++	+	+

Tables VIII and IX show quite clearly that there is no serological relationship between Bacillus S.T., and the sera derived from the following members of the Salmonella group : *Bacillus ærtrycke*, *Bacillus paratyphosus A*, and *Bacillus paratyphosus B*. Furthermore, it can be seen that there is no relationship between Bacillus S.T. and Rettger's ærtrycke strains of *B. anatum* (850 and 851). The third feature demonstrated is that the various strains of Bacillus S.T. (with the exception of P. 1) are almost identical in their serological reactions. Also there appears to be a similarity between Bacillus S.T. and Rettger's non-ærtrycke strains of *B. anatum* C. 1 and C. 5. There is a marked change in the relative behaviour of the antigens of Bacillus S.T. and *Salmonella anatum* in Table VIII and IX. As these tests were done on different occasions, the variations may be attributed either to changes in the agglutinin content of the sera or to the antigenic properties of the emulsion. A statement included in a publication by Edwards and Rettger[2] may explain these irregularities : " It has been stated by Andrewes (1922) that the antigenic characters of the Salmonella group are extremely unstable, and that the antigenic properties of a culture can not be predicted at any time."

TABLE IX.
SERUM DILUTIONS.

Serum.	*Antigen.*	40.	80.	160.	320.	640.	1280.	2560.
Bacillus S.T. (D. 5) after complete immunisation.	Bacillus S.T. (D. 5)...	++	++	++	+	+	—	—
Do.	Bacillus S.T. (D. 2)...	++	++	++	++	++	+	—
Do.	Bacillus S.T. (D. 6)...	++	++	++	++	++	+	—
Do.	Bacillus S.T. (P. 1) ...	+	—	—	—	—	—	—
Do.	Salmonella anatum (Rettger) C. 1.	+	+	+	+	+	+	—
	Salmonella anatum (Rettger) C. 5.	+	+	+	+	+	—	—
Do.	S. anatum (Rettger) ærtrycke type 850.	—	—	—	—	—	—	—
Do.	S. anatum (Rettger) ærtrycke type 850.	—	—	—	—	—	—	—
Salmonella anatum (Rettger) C. 1 non-ærtrycke (after complete immunisation).	Bacillus S.T. (D. 5)...	++	+	—	—	—	—	—
Do.	Bacillus S.T. (D. 2) ...	++	+	—	—	—	—	—
Do.	Bacillus S.T. (D. 6)...	++	+	—	—	—	—	—
Do.	Bacillus S.T. (P. 1) ...	+	—	—	—	—	—	—
Do.	Salmonella anatum (Rettger) C. 1.	++	++	++	+	+	+	—
	Salmonella anatum Rettger) C. 5.	++	++	+	+	+	+	—
Do.	Salmonella anatum ærtrycke type 850.	—	—	—	—	—	—	—
Do.	Salmonella anatum ærtrycke type 851.	+	—	—	—	—	—	—

++ = Precipitate and water clear supernatent fluid. + = Precipitate only.

Bacillus S.T. (D. 5) seems to have produced a much better agglutinating serum than is the case with *S. anatum* (Rettger) C. 1, and, whereas the latter strain does not produce complete agglutination with Bacillus S.T. (D. 5) serum, the Bacillus S.T. (D. 5) does show complete agglutination with *S. anatum* serum.

It is possible that, as mentioned in the pathogenicity experiment, the attenuated state of *S. anatum* may be responsible for this.

The uncertain results which followed our earlier efforts in preparing immune rabbit sera may be accounted for by the known hypersensitiveness to killed cultures of some members of the paratyphoid group.

Summary and Conclusions.

An outbreak of " Keel Disease " in ducklings is described in Britain. It appears to be the first recorded outbreak in this country.

A bacillus belonging to the Salmonella group was apparently the causal organism. This bacillus was found to be culturally, pathogenically, and serologically identical with, or very closely related to, *Salmonella anatum*, Rettger and Scoville, non-ærtrycke type. The organism was not always present in pure culture, but other organisms when present revealed no evidence of pathogenicity. The supply of investigation material was cut short by the subsidence of the outbreak in response to the methods of prevention described, and any further outbreaks which may be brought to our notice will be utilised to investigate further the etiological significance of the Salmonella type of organism isolated.

Our present view is that the pathogenic powers of the *S. anatum* are of a low order, that possibly the organism may be found to be a normal inhabitant of the intestines of ducklings, and, further, that the capacity to become pathogenic depends upon the operation of some predisposing factor, as in this case feeding with fermented foods. Having acquired pathogenic properties, the bacterium rapidly causes an enzootic which is kept in being by the presence of ducklings of the susceptible age. In this outbreak the absence of susceptible ducklings for a period of a few weeks was sufficient, after the removal of the predisposing factor, to stop the outbreak.

No conclusion was reached regarding the deaths in pigeons upon Farm B. The pigeons had not received any of the ducklings' food, and only one dead pigeon was received for examination.

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STRANGLES IN A COUNTRY-BRED REMOUNT DEPOT IN INDIA.

By Major H. Allen, late R.A.V.C.

Introduction.

At the Remount Depot, Mona, Punjab, about 1,800 young stock horses are purchased annually, 80 per cent. from the Punjab.

Half these purchases are sent to Mona Remount Depot and the other half to Sargodha, the parent depot about 40 miles distant.

The stock are purchased at from one to two years of age and remain in the depot till four and a half years of age, when they are issued to the Service.

Each of the depots comprises an area of approximately 10,000 acres, and the average number of young stock horses held is approximately 2,000. There is a similar number of young stock mules, so that each depot holds normally about 4,000 animals plus some 500 bullocks for farm purposes, etc.

The animals are located in "Charges" according to their year of purchase and age, and all animals on entering the depot are segregated in blocks remote from previous years' purchases for a minimum period of six months.

As far as I am aware, in no other depot in the world are so many young stock congregated from different centres at one time in Segregation, and only those who have had to deal with this disease in a country-bred depot in India, on such a scale, can realise the amount of work involved in connection with the control of the disease and endeavours to reduce mortality to a minimum.

Strangles on a large scale must be accepted as inevitable in such a depot in India.

It changes in virulence and incidence from month to month and year to year, and may assume an intense form as the season advances in consequence of exaltation of the virulence of the strains of streptococci or secondary invaders concerned.

A large proportion of the stock may escape without a Strangles lesion. A number develop a cough and laryngitis and nothing more.

Paddock Dispersion System.

In order to control the incidence and virulence of the disease by preventing exaltation of the virus by passage through susceptible animals, the young stock are segregated in units of 12, well apart from one another, and experience has proved that under this system there is a noteworthy diminution in the virulence of the disease.

Coupled with "Paddock dispersion," the following procedure has been adopted :—

(1) The main portion of young stock horses (about 500—600) are received from one breeding area with a view to obviating mixed infection as far as possible. Originally stock were received from five breeding areas, the parent depot situated forty miles from Mona receiving half.

(2) The major purchases are received in the depot as early as possible during the purchasing season (1st September to 31st March of following year), and in large batches.

(3) No stock are sent into the depot after the 31st March.

The largest number of admissions for Strangles as a rule occurs during the end of March, and the incidence of the disease gradually dies down after April.

It has been found by experience that it is a wrong policy to receive young stock during April, as the degree of exaltation of virulence and mortality amongst the cases which do occur is progressive.

(4) As already stated, only 12 young stock horses are put into each paddock.

(5) Each Segregation Block is limited to the number which it can carry, i.e., 240.

(6) Strangles cases are not treated in the Reception Paddocks, but are drafted into special paddocks, isolated at a distance from the reception paddocks, and having Strangles Hospitals attached in which special attention is given to cases requiring individual treatment.

(7) Recovered Strangles cases when returned to Reception Paddocks are kept together and not mixed with unaffected stock.

By this method each block is used to its maximum, whilst each paddock retains its segregation and equalises the degree of infection.

(8) Batches received from Breeding Areas and on different dates are not mixed.

(9) Animals under one year and badly nourished stock are not purchased.

(10) All stock which have contracted Strangles and passed through two or three months' convalescence are drafted into a Semi-Segregation block of paddocks, situated about two miles from the other Segregation blocks, and after two months in this semi-segregation they are drafted into the depot proper, about three and a half miles distant.

Young stock two years and over which have been in segregation for about six months and have not contracted Strangles are similarly treated.

Cases of Strangles which occur in semi-segregation are re-transferred to the original block of segregation.

It is not a sound policy to allow young stock to run the risk of repeated re-infection from recurring cases of Strangles, as their resistance may be unable to withstand the exaltation of the virulence of the disease which occurs in an outbreak.

To prevent this, the animals are moved into large roomy grazing paddocks, which has the added advantage of promoting their growth and development.

Semi-segregation has large "Shaftal" (Persian Clover) and Lucerne Paddocks allotted to them, so that all stock get generous free grazing with an ample calcium content provided.

From 1922 to 1926 there were two large Segregation Blocks constructed on the Cart-wheel plan, i.e., a well in the centre, from which radiated the paddocks.

Stock were received from five breeding areas up to middle of April, and in small batches as a rule.

Paddocks carried 50—60, and each Segregation up to 500 head of stock.

Mules and horses were located in each Segregation block.

Only serious cases of Strangles were removed to Strangles hospital. Tracheotomy had to be frequently performed. Stock from different areas were mixed.

In 1927 two young stock horses Segregation Blocks were divided up into five sections, each section containing four two-and-a-half-acre paddocks, and each separated from others to prevent nose-rubbing. A third segregation block containing twenty-eight four-acre paddocks, which had been put aside for mobilisation purposes, was taken into temporary use for young stock segregation, and allotted to mules.

A Semi-Segregation Block to carry 350 head of stock was constructed during 1928, and put into use that year. It consists of five five-acre dry paddocks and eight grazing paddocks.

During 1926—27 and 1927—28 Strangles seasons the major portion of young stock horses were received from one area.

Only 12 young stock were located in each paddock.

Stock received on different dates were never mixed.

All Strangles and Catarrh cases were removed from reception paddocks on occurrence.

All paddocks were cross tooth-harrowed during Non-Strangles season, and in Strangles season after evacuation.

Special attention was paid to the disposal of manure. All that could not be removed and ploughed into the land was burnt.

Large batches of young stock, 200 and over, were received at times during Strangles seasons 1926-27 and 1927-28.

Each block of Segregation was closed down as far as possible when Strangles started, but this was difficult during 1927 owing to segregation being limited to two blocks.

Tracheotomy has been rarely necessary since 1927.

Sero-vaccine treatment was adopted in the virulent cases during 1926-27, 1927-28, and 1928-29 seasons.

Anti-Streptococcus Serum (Polyvalent) and Vaccines (" Field " mixed autogenous of outbreak in each block of Segregation) were prepared locally.

Some of the stock received may already have been through an attack of Strangles and acquired relative immunity.

The vast majority of deaths are due to Septic Pneumonia.

Smears from the lungs reveal an enormous number of organisms of mixed type. Diphtheroid organisms predominate, but numerous Gram-negative rods, and occasionally chains of micrococci (Streptococci).

Short and concise details of methods of experimental treatment adopted from 1922-23 *Strangles Season to* 1926-27 *Strangles Season :*

1922-23. The vaccine used was prepared as follows :

A mature (but unopened) abscess was selected, and the clean surface was painted with tincture of iodine.

A canula was thrust into the abscess and pus allowed to run direct into a sterile bottle containing beads.

Ether to the extent of 10 to 20 per cent. was added.

Material was allowed to remain in contact with ether for 48 hours and shaken repeatedly.

10% of a 5% Solution of Carbolic acid was added.

The vaccine was then placed in a storage bottle for use when required.

1923-24. The extracted vaccine (Dreyer's method) was prepared by the Laboratory of Veterinary Research, Muktesar.*

The ordinary vaccine was the same as used during 1922-23.

1924-25. Same vaccine as used during 1922-23.

1925-26.

(A) Controls (one third of each batch). They were allowed every opportunity of contracting infection. Temperatures were taken daily after arrival.

When they showed distinct signs of fever it was noted carefully and later blood from the jugular vein was withdrawn, at the rate of 3-6 cc. per pound body weight, into sterile containers.

Blood was allowed to clot, serum withdrawn, and preserved by adding 1 part of 5% carbolic acid to 9 parts of serum (= Preventative Serum).

Blood was withdrawn on the 7th and 14th days after the temperature had come down to normal.

If submaxillary abscesses occurred amongst the controls, pus was withdrawn with sterile precautions, treated with ether, and injected into Lot B.

(B) Pyo-vaccination (one third of each batch).

Injected with etherised pus (presumed to contain " Specific factor ") on arrival.

(C) " Serum—Simultaneous " immunisation.

The stock were injected with 50 c.c. mixed preventative Serum (obtained as indicated above), and one or two days later exposed to infection by smearing nostrils with discharge from nostrils of a number of Strangles " Controls " mixed with a little pus from a freshly opened Strangles abscess.

The above experiments were designed by Dr. J. T. Edwards, the Director of the Imperial Institute of Veterinary Research, Muktesar, and were carried out under his instructions.

1926-27.

The vaccine used was prepared as follows :

The abscess to be punctured was treated with tincture of iodine.

4 c.c. of pus was obtained and transferred to a sterile flask containing beads ; 10 c.c. of sulphuric ether (Merck's) was gradually added and continually agitated.

The material was allowed to stand for twelve hours, with frequent shaking.

5 c.c. of boiled and cooled normal saline solution was added and an almost homogenous mass was thus obtained.

The vaccine thus prepared was kept in a carefully stoppered bottle until required for use.

The results are shown in the Table.

* See paper by Dr. J. T. Edwards in this Journal, Vol. XXXVIII, 1925, p. 256.

TABLE.

Strangles Season.	*No. of Animals Received.*	*No. of Cases of Strangles.*	*No. of Deaths due to Strangles.*	*Percentage of Animals Affected.*	*Percentage of Deaths among Affected Animals.*	*Prophylaxis Adopted.*
1917-18	232	143	6	61·6	4·1	The records for these years are not available.
1918-19	168	85	2	50·5	2·3	
1919-20	223	174	5	78·0	2·2	
1920-21	152	150	—	98·6	—	
1921-22	203	113	3	55·6	2·6	
1922-23	397	243	20	61·2	8·2	Vaccination with pus treated with ether.
1923-24	451	364	31	80·7	8·5	Extracted vaccine (Dreyer's method) and ordinary vaccine.
1924-25	619	522	39	84·3	7·4	Vaccination.
1925-26	558	556	37	99·6	6·6	Pyo-vaccination and Serum Simultaneous.
1926-27	874	341	7	39·01	2·0	Pyo-vaccination and "Paddock dispersion."
1927-28	511	332	17	64·97	5·12	"Paddock dispersion" alone.

CORYNEBACTERIUM PYOGENES AS THE CAUSE OF POLYPOID LESIONS IN THE RECTICULUM OF AN OX.

By G. O. Davies, B.V.SC., M.R.C.V.S., D.V.H.

(*From the Department of Veterinary Pathology, University of Liverpool*).

In the course of a laboratory investigation by the writer into lesions presenting macroscopic actinomycotic characteristics, the material being provided by Mr. H. L. Torrance, M.R.C.V.S., D.V.S.M., the following case was encountered. A general interest and a remote possibility of its association with previous cases stimulated further attention to a possible bacterial cause.

A three-year-old Irish bullock in good condition was slaughtered at the Birkenhead abattoir on 4th February, 1930, and in the course of routine examination a polypoid or granulomatous growth about the size of a billiard ball was found projecting into the lumen from the wall of the reticulum (Fig. 1). Its general appearance led one to suspect a neoplasm, although the presence of small pus points on section cast a doubt on this view. No other lesions were noted in the rest of the body. The material was sectioned, one portion being reserved for histological examination and the remainder for cultural work.

Fig. 1.
The growth attached to the wall of the reticulum.

Histology.

Pieces of tissue were fixed in formalin solution and embedded in paraffin. Sections were prepared for examination and stained by (1) haemotoxylin and eosin, (2) Mallory's eosin and methylene blue, (3) Plaut's stain, (4) haematoxylin followed by Van Gieson's picro-fuchsin, and (5) Loeffler's alkaline methylene blue.

Microscopical examination of the sections shewed the main

composition of the lesion to be granulation tissue. Scattered throughout this tissue were small abscesses, consisting chiefly of polymorphonuclear leucocytes, and in the majority of these were to be found colonies somewhat resembling those associated with actinomycotic lesions. This similarity was only apparent, even with a low power objective, as a close examination revealed the complete absence of the characteristic peripheral zone of clubs.

The colonies, which stained uniformly throughout, were not of any constant shape and consisted of masses of bacillary organisms. In the larger colonies the centre was devoid of structure, with the organisms becoming more numerous towards the boundary whereas, in the smaller, the organisms were to be found more or less evenly distributed throughout, although in all cases they appeared in greater numbers at the periphery.

Immediately surrounding one or more of these colonies was a dense mass of leucocytes, the majority being polynuclear. This zone was very definitely defined and in some cases had retracted from the surrounding granulation tissue, probably as a result of the fixation of the tissue.

The granulation tissue shewed variations in cellular structure as one progressed from within outwards. The portion immediately surrounding the round cells was characterised by large numbers

FIG. 2.
a, Colony of bacilli ; *b*, Zone of leucocytes ; *c*, Zone of granulation tissue.

of plasma cells scattered amongst the fibres of connective tissue. They became progressively fewer as the fibrous tissue elements became more prominent. An occasional giant cell was also seen.

The photomicrograph (Fig. 2) shews clearly the three defined areas as seen with a lower power objective. The central colony is embedded in a mass of leucocytes and surrounded by granulation tissue.

Cultural Examination.

The surface of the lesion was seared with a spatula and incisions were made into its substance with sterile instruments. On squeezing a little of the tissue with forceps, pus-points about a pin head in size appeared on the surface. A cursory examination for granules was made, but without success. Cultures were attempted on agar, nasgar, bouillon, and deep glucose agar. When these were examined on the third day all were apparently sterile with the exception of two nasgar media. These presented a few fine colonies of a streptococcal nature. Films were prepared, and whilst, at first, it was thought they were streptococci, a further and closer study revealed a pleomorphic organism with a general cocco-bacillary appearance, and a few curved and club-formed short bacilli. Nothing further was done with these cultures. On the eleventh day following inoculation, the glucose agar shake cultures were examined, and numerous fine colonies were apparent throughout the medium. The *Streptothrix actinomyces* was immediately suspected, but films made from a number of colonies shewed them to resemble those grown aerobically, cocco-bacillary forms predominating, with occasional clubs and curved forms. Subcultures were made and grown under both aerobic and anaerobic conditions. The organism grew best on blood agar and appeared to grow equally well both aerobically and anaerobically. Haemolysis was evident in all cultures. Nasgar did not appear to answer the requirements of the organism as well as blood-agar, some tubes remaining sterile, whereas others only had a very fine growth after four days' incubation. At this stage the organism seemed to assume more definite bacillary forms and occasional fields were diphtheroid in appearance. Cultures on Loeffler's serum gave a characteristic pitted appearance after 24 hours' incubation. Milk was clotted in 24 hours, and as incubation continued a gradual digestion of the clot was evident. The organism grew well in serum bouillon, forming a heavy sediment, together wtih a few fine colonies which adhered to the sides of the tube. The medium itself remained perfectly clear. A similar growth was obtained in serum gelatine at 37° to 38° C. and the medium did not solidify on standing at room temperature. The organism did not grow very well on glycerine egg medium, but fine colonies appeared to be growing in a trench which marked the needle track, indicating liquefaction as in the case of solid serum.

Fermentation reactions were studied in Hiss's serum water. Acid production was not very marked for several days and gas was absent in all sugars. Glucose, Laevulose, Lactose, Maltose, Arabinose, Xylose, and Galactose were fermented, but the following

were negative: Saccharose, Mannite, Salacin, Sorbite, Inulin, and Inosite.

The above characteristics led one to conclude that the isolated organism was *Corynebacterium pyogenes*.

Discussion.

B. pyogenes has long been known to be associated with suppurative processes in domestic animals. Magnusson (1) claims to have isolated the organism in ten cases of bovine actinomycosis, representing 8% of all his recorded cases of actinomycosis from this and other causes. Hülphers (2) records *B. pyogenes* as being the casual organism of actinomycosis in cattle in 19 cases out of 136, and Gunst (3) in 4 cases out of 125. Of all the above cases only Magnusson records its presence in cases other than in the jaw. He dealt with one case in the rumen and four in the reticulum, all following traumatic injuries. The former case is of particular interest, as the reproduced photograph of the case bears a striking similarity to the one which is recorded here. Magnusson states: "The microscopic picture of both crushed granules and on section is the same as that of actinomycosis in the jaw caused by *Streptothrix Israeli*. If *B. pyogenes* alone can cause granules with clubs and give rise to actinomycosis, then I consider it to be impossible to differentiate such a process from Streptothrix infection by microscopic examination alone." Whilst there is a very marked resemblance between his case and the one here described, the above observations are not borne out. Low power examination of a section may shew a slight resemblance to actinomycosis, but a closer study reveals the complete absence of peripheral clubs, a fact which is demonstrated very clearly by appropriate staining. Granules were not found in the pus, but a great deal of importance cannot be attached to this, as the search could not be considered exhaustive. Nevertheless, it is conceivable that in Magnusson's cases, as he himself suggests, there was an underlying Streptothrix infection masked by a heavy secondary infection by *B. pyogenes*, which would probably account for the granules and clubs.

There was no apparent evidence in this case of any injury to the reticulum.

Conclusions.

Corynebacterium pyogenes was isolated in pure culture from a granulomatous lesion in the reticulum of an ox.

The colonies present in the lesion were distinguishable microscopically from the colonies associated with actinomycosis.

The writer wishes to express his thanks to Mr. H. L. Torrance, M.R.C.V.S., D.V.S.M., for the material which has been the subject of this investigation, and also to Professor S. H. Gaiger, F.R.C.V.S., for helpful advice and criticism.

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ACTIVE IMMUNISATION OF FOWLS AGAINST FOWL PLAGUE.

By Harvey S. Purchase, B.Sc., M.R.C.V.S.,

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I. Experiments with a Phenol-Glycerine Vaccine.

Immunisation of fowls against Fowl Plague has been admitted by most workers to be a most difficult problem. Various methods and principles have been tried with very unconvincing results, and none has been found sufficiently reliable to warrant use in large flocks against a natural infection in countries where the disease is enzootic.

In 1928, Todd (1), working in London, published encouraging results obtained by a phenolised virus-vaccine, having previously failed to obtain any immunity with heat or formalin-treated virus. The method was described as having "constantly produced a solid immunity against the disease in fowls." One batch of vaccine was reported to have failed to produce any immunity, and in this instance the failure was considered to be due to imperfect emulsification.

Encouraged by these results, and working with a number of strains for cross-immunity tests, attempts were made to obtain immune birds by Todd's method.

Technique.

The first batch of vaccines was made late in January, 1929, carefully carrying out the details of technique described by Todd. The livers used were from carcases of birds just dead of Fowl Plague, and were aseptically removed; the edges only of the livers were used, thus avoiding the large vessels which are situated in the centre of each lobe. This precaution, which is advocated by Todd, avoids excess of virulent blood. The liver material was then weighed and ground up with broken glass which had been splintered by immersing heated glass-tubing in sterile water, to ensure thorough laceration of the tissue. To this ground liver material was added four times its weight of glycerine-phenol mixture, made as follows :—

Glycerine	60·0 c.c.
Phenol	0·5 gm.
Distilled neutral water	39·5 c.c.

The emulsions were then passed through a sterile copper gauze sieve having 16 meshes per centimetre. The phenol used was from a freshly-opened bottle of crystallised pure carbolic acid. The vaccines thus prepared were stored in glass-stoppered bottles in the dark at room temperature (14°–21° C.). They were shaken two or more times a day for seven days, and then placed in the cold store. The temperature in the cold store varied from — 2° to + 5° C.

Experiment I.—The first experiment was made with two groups of five fowls, using two vaccines, each made from a different strain of virus. Vaccine I was made with a strain of virus obtained from Palestine, through the courtesy of Major J. M. Smith, C.V.O. The strain used by Todd was isolated by Rice[2], and obtained from the Ministry's laboratory, and is thus from the same original outbreak of Fowl Plague. Of these ten fowls not a single one survived the initial dose of 0·5 c.c. intravenously.

VACCINE I.

PALESTINE VIRUS, MADE JANUARY 27th, 1929.

Date 1929	*No. of Fowl*	*Age of Vaccine*	*Dose*	*Result*	*Method of Injection*
5/2	731	9 days	0·5 c.c.	Died 3 days	I/V
5/2	777	9 days	0·5 c.c.	Died 3 days	I/V
5/2	779	9 days	0·5 c.c.	Died 4 days	I/V
5/2	793	9 days	0·5 c.c.	Died 5 days	I/V
5/2	704	9 days	0·5 c.c.	Died 2 days	I/V
11/2	560	15 days	0·5 c.c.	Died 3 days	I/V

Put in the cold store February 5th, 1929.

VACCINE A.

RICE VIRUS MADE JANUARY 27th, 1929.

Date 1929	*No. of Fowl*	*Age of Vaccine*	*Dose*	*Result*	*Method of Injection*
5/2	789	9 days	0·5 c.c.	Died 7 days	I/V
5/2	791	9 days	0·5 c.c.	Died 9 days	I/V
5/2	780	9 days	0·5 c.c.	Died 3 days	I/V
5/2	788	9 days	0·5 c.c.	Died 6 days	I/V
5/2	785	9 days	0·5 c.c.	Died 1½ days	I/V
11/2	2168	15 days	0·5 c.c.	Died 2 days	I/V

Put in the cold store February 5th, 1929.

Experiment II.—This vaccine was prepared by the same method as the preceding, and was found to be virulent after seven days at room temperature, although the bird took five days to succumb.

VACCINE II.

PALESTINE VIRUS MADE FEBRUARY 8th, 1929.

Date 1929	*No. of Fowl*	*Age of Vaccine*	*Dose*	*Result*	*Method of Injection*
11/2	1927	3 days	0·5 c.c.	Died 2 days	I/M
13/2	2220	5 days	0·5 c.c.	Died 5 days	I/M
15/2	2354	7 days	0·5 c.c.	Died 10 days	I/M (F.P. +)
18/2	799	10 days	0·5 c.c.	Died 4 days	I/V
20/2	832	12 days	0·5 c.c.	Died 4 days	I/V
22/2	798	14 days	3 doses	Immunised	I/V

Put in the cold store February 15th, 1929, till February 18th, 1929. At the laboratory temperature February 18th, 1929, to February 20th, 1929. Cold store February 20th, 1929.

Fowl 2354 did not die of the usual acute form of fowl plague, but proof inoculation showed the presence of the normal virus. Finally, fowl 798

was immunised after receiving three successive doses of 0·5 c.c., 1·0 c.c., and 2·5 c.c. of this vaccine at weekly intervals. The test dose in all these experiments consisted of 0·25 c.c. of an emulsion made by grinding a piece of virulent liver or spleen the size of a pea in 5 c.c. of saline. This was injected intramuscularly, always using a healthy fowl as a control to the bird to be tested. The control bird succumbed in two days.

Experiment III.—As a result of a personal communication from Dr. Todd, all subsequent inoculations were carried out by the intramuscular route. In a later paper he[2] remarks that the intravenous route is probably less (certainly not more) effective than intramuscular inoculation in the case of fowls inoculated with fowl plague virus. In view of these findings, the fatal results of inoculating the vaccine by the intravenous method could not have been due to that channel. This is emphasised by the results obtained with Vaccine III. Fowl 650, although it withstood a test dose fatal to a control bird in fifty hours, succumbed two months later on testing with a different strain of virus.

VACCINE III.

RICE VIRUS MADE FEBRUARY 22nd, 1929.

Date 1929	*No. of Fowl*	*Age of Vaccine*	*Dose*	*Result*	*Method of Injection*	*Immunity Tests*
25/2	830	3 days	0·5 c.c.	Died 2 days	I/M	—
22/2	863	3 days	0·5 c.c.	Died 2 days	I/M	—
28/2	851	6 days	0·5 c.c.	Died 3 days	S/C	—
7/3	878	13 days	3 doses	Survived	I/M	Nil
17/4	692	23 days	3 doses	Survived	I/M	Nil
17/4	650	23 days	3 doses	Survived	I/M	Immune

Placed in the cold store March 1st, 1929, and kept there.

Experiment IV.—The apparently erratic results that may be obtained while using this method are well demonstrated by the following table of Vaccine X. The birds were given three successive doses of 0·5 c.c., 1·5 c.c., and 3·0 c.c., at seven days intervals, and then tested a week after the third dose, using also a control bird, which invariably succumbed well within the normal period for fowl plague.

The Ex virus was isolated from an important outbreak on a large poultry farm in the South-East of England by Mr. N. H. Hole of this laboratory during routine diagnosis.

VACCINE X.

EX VIRUS, MADE MARCH 21st, 1929.

Date 1929	*No. of Fowl*	*Age of Vaccine*	*Dose*	*Result*	*Method of Injection*	*Immunity Tests*
28/3	1027	7 days	0·5 c.c.	Died 2 days	I/M	—
28/3	1031	7 days	0·5 c.c.	Died 2 days	I/M	—
2/4	943	12 days	0·5 c.c.	Died 3 days	I/M	—
4/4	974	14 days	3 doses	Died 3rd dose	I/M	—
9/4	930	19 days	3 doses	Survived	I/M	Nil
9/4	1003	19 days	2 doses	Died 3 days	I/M	—
26/4	1089	36 days	3 doses	Survived	I/M	Immune
26/4	1091	36 days	3 doses	Survived	I/M	Immune

Put in the cold store March 28th, 1929, till March 30th, 1929. At the laboratory temperature March 30th, 1929, to April 4th, 1929. In the cold store April 4th, 1929, onwards.

Experiment V.—In subsequent attempts at immunising fowls by this method the vaccine was stored, after seven days at room temperature, in an ice box at from −3° to −10° C. This made little or no difference to the results obtained, as is shown by the following protocols.

VACCINE XI.
PALESTINE VIRUS, MADE APRIL 12th, 1929.

Date 1929	*No. of Fowl*	*Age of Vaccine*	*Dose*	*Result*	*Method of Injection*	*Immunity Tests*
15/4	898	3 days	0·5 c.c.	Died 3 days	I/M	—
17/4	678	5 days	0·5 c.c.	Died 3 days	I/M	—
30/4	1096	18 days	0·5 c.c.	Died 3 days	I/M	—
7/5	1128	25 days	3 doses	Survived	I/M	Immune
13/5	1147	31 days	3 doses	Survived	I/M	Nil
13/5	1186	31 days	3 doses	Survived	I/M	Nil

Put in the ice box April 19th, 1929, and kept there.

In Vaccine XII the Bax virus was used, which had been recently isolated from an outbreak of fowl plague in the South of England. Only a few fowls were kept on this establishment. It was isolated through the diagnosis department of this laboratory by Mr. N. H. Hole, M.R.C.V.S.

VACCINE XII.
BAX VIRUS, MADE APRIL 18th, 1929.

Date 1929	*No. of Fowl*	*Age of Vaccine*	*Dose*	*Result*	*Method of Injection*	*Immunity Tests*
26/4	1074	8 days	0·5 c.c.	Died 2 days	I/M	—
30/4	1097	10 days	0·5 c.c.	Died 3 days	I/M	—
7/5	1154	17 days	3 doses	Survived	I/M	Immune
13/5	1146	23 days	3 doses	Survived	I/M	Nil
13/5	1178	23 days	3 doses	Survived	I/M	Immune
16/5	1180	26 days	3 doses	Survived	I/M	Nil
16/5	1164	26 days	3 doses	Survived	I/M	Nil
30/5	1275	40 days	3 doses	Survived	I/M	Nil
30/5	1282	40 days	3 doses	Survived	I/M	Nil
11/6	1277	52 days	3 doses	Survived	I/M	Immune
11/6	1255	52 days	3 doses	Survived	I/M	Immune

Put in ice box April 26th, 1929, to April 30th, 1929. At laboratory temperature April 30th, 1929, to May 7th, 1929 ; in the ice box May 7th, 1929.

Experiment VI.—The following two batches of vaccine were made during the warmer months, and the room temperature varied from 16° to 23° C. This may account for the more rapid devitalisation of the viruses.

The Egyptian Virus was obtained through the courtesy of M. Lagrange[4] of the Consel Sanitaire, Maritime et Quarantenaire, d'Egypte

VACCINE XIII.
EGYPT VIRUS, MADE MAY 21st, 1929.

Date 1929	*No. of Fowl*	*Age of Vaccine*	*Dose*	*Result*	*Method of Injection*	*Immunity Tests*
25/5	1206	4 days	3 doses	Survived	I/M	Nil
29/5	1278	8 days	2nd dose	Died 4 days	I/M	—
29/5	1252	8 days	3 doses	Survived	I/M	Nil
11/6	1259	22 days	3 doses	Survived	I/M	Nil
11/6	1304	22 days	3 doses	Survived	I/M	Nil

Put into ice box May 27th, 1929.

VACCINE IV.

Ex-Virus, made May 21st, 1929

Date 1929	*No. of Fowl*	*Age of Vaccine*	*Dose*	*Result*	*Method of Injection*	*Immunity Tests*
25/5	1203	4 days	2nd dose	Died 3 days	I/M	—
29/5	1247	8 days	3 doses	Survived	I/M	Nil
29/5	1292	8 days	3 doses	Survived	I/M	Nil

Placed in the ice box May 29th, 1929.

To rule out bacterial contamination as a possible cause for the inefficacy of the vaccines, special aseptic precautions were taken during the making and using of the vaccines. This was followed by cultural examinations of the emulsions for any bacterial growth.

Two agar plates were thickly smeared with material from each vaccine, and a tube of peptone broth was inoculated from each sample.

Vaccine	*Agar Plate Sown* 16/7/29	*Peptone Broth Sown* 16/7/29
XI	One colony 18/7/29	No growth 22/7/29
XII	No growth 22/7/29	No growth 22/7/29
XIII	No growth 22/7/29	No growth 22/7/29
XIV	No growth 22/7/29	No growth 22/7/29

The tubes and plates were incubated aerobically at 37° C. for two days, and then examined for any growth. Further incubation and examination of the plates and tubes over a number of days was negative.

Summary.

Of 46 birds used for immunisation experiments, 19 survived the three inoculations, and of these only nine (20 per cent.) were found immune after a test dose of virus given seven days after the last dose of vaccine.

In the experiments described it usually required more than seven days in the dark at room temperature, at approximately 21° C., before a vaccine prepared by Todd's technique lost its virulence in half cubic centimetre doses given intramuscularly to healthy susceptible fowls.

II. The Use of Liver Material for the Preparation of Vaccines.

Todd[1] records his inability to obtain a satisfactory vaccine by using the cellular layer of centrifuged blood as the source of virus, and suggests that hæmoglobin or some other constituent of the blood may prevent the action of the phenol on the virus. He found the mixture was apparently as virulent after storage for fifty-two days at 20° C. as when first made. It would appear, however, from the

following results that the action of the phenol is probably dependent not so much on the absence of hæmoglobin as on the presence of bile.

Technique.

The virulent material consisted of the pectoral muscles of two fresh carcases of birds recently dead from typical fowl plague (Palestine strain). This muscle was chosen as the infective material for these experiments because of the small amount of blood or hæmoglobin it contained.

One emulsion was made by using this flesh in place of virulent liver material, but the grinding process was more tedious and very troublesome owing to the tenacity of the muscle fibres as compared with the liver tissue. Similarly, two other quantities of muscles were weighed, to one of which was added an equal weight of fresh *healthy* liver, and to the other 1 c.c. of fresh bile from a healthy fowl. These were ground and sieved, the required amount of Todd's phenol glycerine fluid added, and the emulsions placed in stoppered bottles. The three stoppered bottles were kept at room temperature for eight days and then transferred to cold store. They were always placed close together so that all external conditions might be as similar as possible. From time to time the virulence was tested by intramuscular injections of 0·5 c.c. of the emulsions after shaking the bottles well.

The three emulsions showed well-marked differences with respect to the times required to inactivate the virus, as is shown by the results to follow.

All were prepared on March 6th, 1929, and left in a darkened drawer at room temperature for eight days ; placed in the cold store March 14th, 1929.

Those birds which did not succumb to the injection of 0·5 c.c. of the emulsions were given two further doses of 1 c.c. and 2 c.c. respectively, all doses being given at weekly intervals. They were then tested for immunity by a small dose of virus ten days after the last injection of emulsion. In no case could any appreciable degree of immunity be demonstrated.

Emulsions were prepared as follows :—

Emulsion I—	
Virulent muscle	5·8 gm.
Glycerine Phenol	23·2 gm.
Emulsion II—	
Virulent muscle	6·6 gm.
Healthy liver	6·6 gm.
Glycerine Phenol	26·4 gm.
Emulsion III—	
Virulent muscle	7·0 gm.
Healthy bile	1·0 c.c.
Glycerine Phenol	28·0 gm.

	Emulsion I.			*Emulsion II.*		*Emulsion III.*	
Date 1929	*Age of Emulsion*	*No. of Fowl*	*Result*	*No. of Fowl*	*Result*	*No. of Fowl*	*Result*
7/3	1 day	2209	Died 3 days	860	Died 2 days	877	Died 4 days
14/3	8 days	654	Died 4 days	730	Died 4 days	657	Nil. No Immunity
26/3	20 days	978	Died 5 days	1038	Nil. No Immunity	1012	Nil. No Immunity
4/4	29 days	923	Died 6 days	941	Nil. No Immunity	963	Nil. No Immunity
11/4	34 days	900	Died 3 days				
30/4	53 days	1103	Nil				
6/5	60 days	1103	1 c.c. Died 4 days				
13/5	67 days	1187	Nil				
21/5	75 days	1187	1 c.c. Died 3 days				
24/5	78 days	1198	Received 2 c.c. Nil				
31/5	—	1198	0·25 c.c. virulent blood, died 4 days				

Conclusions.

In these parallel experiments, using virulent fowl plague muscle, it was found that normal liver material hastened markedly the devitalisation of the virus, and that this was probably due to its bile content as the addition of bile was found to be even more effective. In these experiments phenol glycerine fluid was used in the same proportions to the virulent material as in the vaccine preparations.

References.

[1] Todd. Br. Jn. Exp. Path., ix, 1928, p. 101.
[2] Rice. Vet. Rec., iv, 1924, p. 207.
[3] Todd. Br. Jn. Exp. Path., ix, 1928, p. 244.
[4] Lagrange. Bull. de la Soc. Path. Exo., xxii, 1929, p. 64.

ON CERTAIN PATHOLOGICAL CONDITIONS IN A TIGER.

By Drs. H. H. Curson and A. D. Thomas,
Veterinary Research Laboratories Pretoria, S.A.

On November 1st, 1929, the skinned carcase (minus head and claws) of a Bengal tigress was received at this Institution from Dr. Bigalke, Director of the Zoological Gardens, Pretoria. The animal was purchased on May 14th, 1914, and had therefore been in captivity for at least 15 years. Although lame in the right foreleg for many years, the tigress was destroyed on account of loss of condition and unsteady gait. It is believed that the lameness was directly due to a bite inflicted by a male companion during 1914.

Upon post-mortem examination (P.M. 278, of November 1st, 1929) the following pathological conditions were observed :—

(*a*) Chronic arthritis of the right shoulder joint, involving marked atrophy of the right scapula. The scapula in its long axis was approximately 2 cm. shorter than the same bone of the opposite limb. This was due to the absence of the neck in the glenoid angle, the articular surface being in closer relation with the main body of the bone. As a result, the acromion projected 1·5 cm. beyond the articular surface. The glenoid surface, instead of being concave, was convex, and at its postero-lateral aspect there was erosion of the cartilage. The deformity also included the absence of the *tuber scapulæ* and its medial coracoid process. The humerus, instead of presenting a convex articular head, showed a concavity corresponding to the articular surface of the scapula. On this surface there was also marked erosion of the cartilage. The bicipital groove at its upper aspect and for a distance of 5 cm. was filled with a rugged uneven exostosis, from which fragments of bony tissue projected. Both medial and lateral tuberosities also showed lesions characterised by erosion of the bone and formation of jagged fragments of osseous tissue in the neighbourhood of the joint. Extending medially around the margin of the joint was an irregular dumb-bell shaped mass composed of jagged pieces of bone varying in size from a pin's head to a hen's egg. The " grip " of the dumb-bell was disposed medially, with the enlarged ends cranial and caudal to the shoulder joint. The atrophy so noticeable in the scapula was less marked in the right humerus (see Fig. 1). It is difficult to state how the lesion originated, but it would appear that the bite referred to was in some way responsible, either by causing a fracture or by initiating a chronic arthritis.

(*b*) Both ovaries were cystic. The right gland, measuring 3·5 cm. by 2·5 cm., showed a cyst 2 cm. in diameter, and the left, 2·5 cm. by 2 cm. in size, contained two small cysts each approximately 0·4 cm. in diameter. Neither ovary showed changes suggesting recent ovulation. The cysts contained a clear watery fluid (see Fig. 2).

(*c*) In both lungs there were present about half a dozen firm smooth nodules, varying in size from that of a pea to that of a hen's egg. These were either embedded deeply in the lung substance

FIG. 1.
Lateral Aspects of right Scapula and head of humerus.

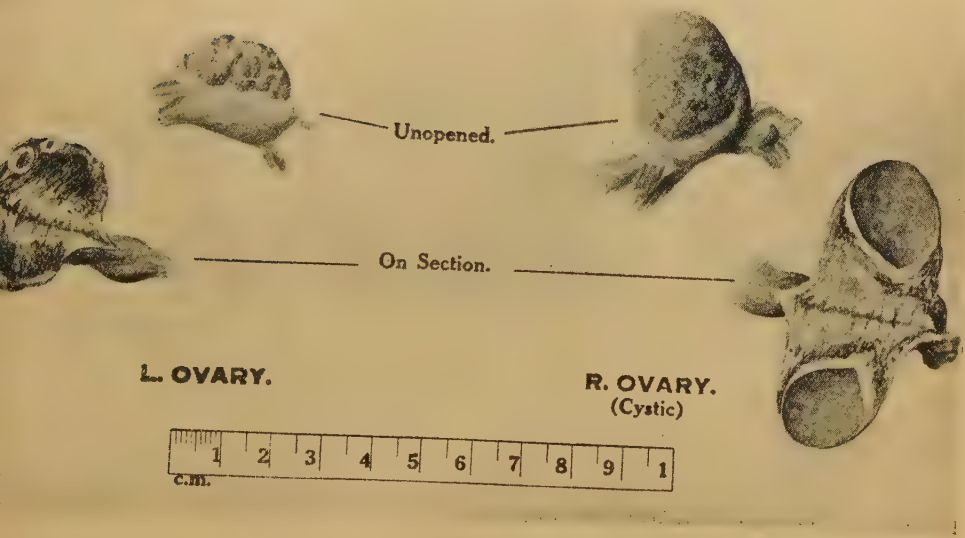

FIG. 2.

or bulging and covered by pleura. Upon incision, the largest was found to contain a quantity of brown, viscid, partly coagulated substance. The wall of the nodules consisted of irregularly lobulated, light pinkish-grey tissue, in parts mottled and having a distinct resemblance to thyroid tissue. This was particularly evident in the smaller nodules in which less fluid substance was present. The lungs otherwise were normally elastic, but showed a greyish punctiform pigmentation (anthracosis).

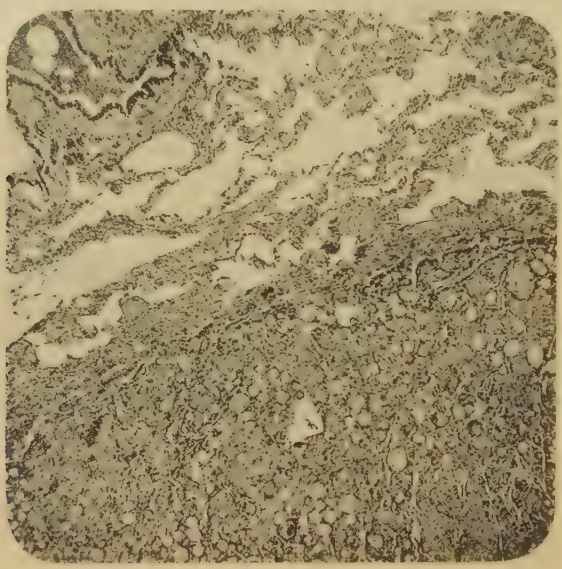

FIG. 3.
Section from one of the Lung Nodules, X 40.

Microscopic examination (see Fig. 3) of sections from these nodules revealed a tissue remarkably like thyroid, *i.e.*, round and oval acini, lined by low cuboidal epithelium and filled with a smooth hyaline or colloid matter. The acini were very irregular and unevenly distributed, some even tending to become cystic. The cellular portion in parts was greatly increased, consisting of solid masses of closely packed epithelial cells, somewhat resembling the so-called *Struma parenchymatosa*. Numerous blood vessels were present in the stroma, and in many instances small hæmorrhages had taken place into surrounding acini. This thyroid-like tissue was fairly sharply demarcated from the lung. There was no definite fibrous capsule, but only a zone of collapsed and compressed alveoli. The thyroid gland itself was unfortunately destroyed, and thus could not be examined. There is little doubt, however, that the nodules represent metastases from an adeno-carcinoma of the thyroid. Disturbances of the thyroid, including new-growths, are not rare in captive wild carnivores.*

The intestinal parasites collected included two specimens of *Anchylostoma caninum* (a male and female) and several specimens of *Toxocara mystax*.

* Fox, H. (1923). " Disease in captive wild mammals and birds." J. B. Lippincott Co., London. p. 333.

FOWL PARALYSIS IN ENGLAND.

In a communication entitled "Preliminary Report on an Outbreak of Fowl Paralysis in England," published in this journal, Volume XLII, p. 63-76, March, 1930, by us, it was stated that this disease had hitherto not been recorded as occurring in the British Isles. We have discovered that this statement is not correct, as Mr. I. A. Galloway, B.Sc., M.R.C.V.S., of the National Institute for Medical Research, mentioned the disease in a paper entitled "Encephalomyelitis of Man and Animals," published in the Proceedings of the Royal Society of Medicine, volume XXII, part 2, p. 1167, in March, 1929. He stated that he had recently encountered this disease in England and had been able to transmit it to healthy susceptible fowls by the intracerebral inoculation of emulsions of affected nerve tissue.

At the time of publication of our communication we were unaware of Mr. Galloway's paper and we wish to offer apologies for our error.

C. A. McGaughey,
A. W. Downie.

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